Norwegian Journal of Agricultural Sciences

Vol. 3 1989 No. 2

Norwegian Agricultural Advisory Centre, Ås, Norway

NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES

Norwegian Journal of Agricultural Sciences fills a need created by the folding of Scientific Reports of the Agricultural University of Norway and Research in Norwegian Agriculture for a forum for publishing Norwegian research with international interest within the following areas: Aquaculture, Animal Science, Soil Science, Agricultural Engineering and Technology, Natural Resources and Environment, Food Technology, Crop Science, Forestry, Economics and Society Planning.

PUBLISHER

The journal is published by Norwegian Agricultural Advisory Centre, Moerveien 12, N-1430 Ås, Norway. Norwegian Journal of Agricultural Sciences (ISSN 0801-5341) is published four times a year, each issue containing approximately 100 pages. Four issues comprise one volume. Annual subscription NOK 400,–. Subscribers receive supplement issues free of charge, but these can also be ordered separately through the publisher.

CORRESPONDENCE

All Correspondande, editorial or otherwise, should be adressed to Norwegian Agricultural Advisory Centre.

The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

ISSN 0801–5341

IRRIGATION OF CEREALS, POTATO, CARROT AND ONION ON A LOAM SOIL AT VARIOUS LEVELS OF MOISTURE DEFICIT

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Riley, H. 1989. Irrigation of cereals, potato, carrot and onion on a loam soil at various levels of moisture deficit. Norwegian Journal of Agricultural Sciences 3:117-145. ISSN 0801-5341.

Field trials were performed on a morainic loam soil to determine optimum irrigation strategy in cereals, potato, carrot and onion. Irrigation was given at deficits of 20, 40 and 60 mm, calculated using a water balance model. Rooting depth was estimated to be 60 cm and the available water capacity (AWC) was found to be 95 mm. Regression equations were derived of relative yield on relative evapotranspiration at different growth stages. Effects on various irrigation strategies on yields, water requirements and drainage losses were predicted over 25 years. Mean maximum responses to irrigation at an AWC of 110 mm were about 20% for potato and carrot and 12% for cereals and onion. At 70 mm AWC the figures were approx. 40% and 20%. Non-irrigated yields were greater than 95% of those with irrigation in half or one-third of all years, and lower than 75% in about one-quarter or one-third on all years, at 110 mm and 70 mm AWC, respectively. Irrigation at 50% depletion of AWC gave only slightly lower yields than more frequent irrigation, and considerable reductions in both the amounts of water required and in drainage losses.

Key words: Carrot, cereals, drainage, evapotranspiration, irrigation strategy, loam, onion, potato, soil moisture deficit.

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The extent to which soil moisture reserves may be depleted before yields are affected may be expected to vary both with crop type and with meteorological conditions. It is commonly assumed that irrigation is required when half to two-thirds of the plant available water in the root zone has been used (Aslyng and Hansen 1982, Gregersen and Olesen 1980, Johansson 1974).

In Danish investigations, irrigation has been found to be profitable at ca. 25% soil moisture depletion in potatoes, at 25-50% in ryegrass whilst in barley depletion could reach 60% before irrigation became necessary (Jørgensen 1980, 1984a,b). Wolf (1984) presented similar results for cereals and potatoes in Northern Germany. Knowledge of acceptable levels of soil moisture depletion is desirable, both for economies in labour input and in water use, and for the avoidance of leaching due to rainfall after irrigation.

A crucial consideration in this matter is to determine the amount of water available to plants within the root zone. This varies with both root depth and root density (Madsen and Platou 1983).

This paper contains the results of experiments performed during 1985-1987 at Kise Research Station (60° 47'N 10°

49'E, 135 m asl) to determine optimum irrigation strategy on a morainic loam soil. This soil is typical for the most commonly irrigated areas in the region of Cambro-Silurian deposits bordering Lake Mjøsa.

METHODS AND MATERIALS

Experimental design

The effects of irrigation at three levels of soil moisture depletion (A = <20 mm, B = 35-45 mm and C = 55-65 mm), in addition to a treatment with precipitation only (D), were compared over three years in wheat, barley and potato and over two years in carrot (factory) and onion (grown from sets). Three replicates were used in all cases. Irrigation was given on 4 x 4 m plots using specially constructed shielded sprinkler wagons. Crops were grown in pairs (barley/wheat, early and late maincrop potato and onion/carrot), such that crops in each pair received irrigation simultaneously.

Irrigation was only given at the growth stages which have previously been shown to be most susceptible to drought in the region (Dragland 1974, 1978a,b, 1979, 1985):
Cereals: From tillering (Zadoks 20) to anthesis (Zadoks 65). Potato (maincrop): From tuber formation (3 weeks from emergence) until harvest.Carrot/onion: From early July (ca. 5 weeks from sowing/planting) until harvest. Though some crops may require irrigation at planting (notably onion) in order to achieve satisfactory establishment, this was not necessary in any of the trial years.

In order to achieve the desired moisture deficits at such stages, treatments B and C were sheltered from rainfall for varying periods using 5x4 m moveable covers of 0.15 mm thick clear polythene. The covers allowed free passage of air above the crop, and have previously been found to have little effect on mean air temperature (Dragland 1974). Treatments A and D were unsheltered.

Estimation of soil moisture deficits

The water balance model of Kristensen and Jensen (1975) was used, employing

Table 1. Climatic conditons at Kise in the trial years compared with long-term mean values

	Year	May	June	July	August	Sept.	May-Sept.
Air temp. (C)	1985	8.2	13.2	15.0	13.5	8.5	11.7
	1986	8.8	14.9	15.4	11.9	7.9	11.8
	1987	7.7	10.8	15.0	11.8	8.7	10.8
	Normal[1]	8.2	13.5	15.2	14.1	9.7	12.2
Pot.evap. (mm)	1985	50	70	66	51	34	271
	1986	50	80	97	46	42	315
	1987	61	40	81	48	39	269
	Normal[2]	61	84	82	68	40	335
Precipitation(mm)	1985	20	69	95	127	124	434
	1986	34	39	29	127	36	266
	1987	69	179	49	101	100	499
	Normal[2]	45	65	63	67	65	305

[1]) 1951 - 1980 [2]) 1963 - 1987

standard values for the establishment of adequate plant cover for full transpiration, which corresponds to a leaf area index (LAI) of 2.8-3.0 (Kristensen 1974, Ritchie 1972). This stage was reached three weeks from emergence in the case of cereals and potato, and ten weeks from sowing/planting in the case of carrot and onion. Corrections for measured values were made in some cases.

Neutron-probe measurements (see Appendix I) of soil moisture were made regularly on two replicates of all treatments, and were used to correct irrigation scheduling in 1987, when extremely high rainfall caused seepage onto sheltered plots.

Weather conditions and amounts of irrigation

Temperature, potential evaporation and rainfall data are shown in Table 1. The evaporation data are from the «Thorsrud

Table 2. Dates of sowing, planting, emergence, sheltering and irrigation, together with irrigation quantities

Year	Sowing/ planting	Emergence	Sheltering	Trt.	Irrigation dates and amounts (mm)		
CEREALS							
			None	A	14.6(15)	20.6(15)	
1985	14.5	22.5	31.5-5.7	B	20.6(35)	9.7(25)	
			» - »	C	5.7(60)		
			None	A	20.6(20)	27.6(20)	3.7(20)
							15.7(20)
1986	13.5	24.5	6.6-15.7	B	23.6(40)	3.7(40)	15.7(40)
			» - »	C	30.6(60)	15.7(60)	
			None	A	9.7(10)	15.7(20)	22.7(20)
1987	7.5	20.5	3.6-5.8	B	15.6(50)[1]	15.7(40)	
			» - »	C	15.6(50)[1]	30.7(60)	
POTATO							
			None	A	1.8(20)		
1985	14.5	30.5	21.6-23.7	B	9.7(40)	30.7(40)	
			» - »	C	24.7(65)	1.8(20)	
			None	A	24.6(20)	2.7(20)	10.7(20)
						16.7(20)	23.7(20)
1986	14.5	15.6	18.6-30.9	B	3.7(40)	16.7(40)	29.7(40)
							26.8(40)
			» - »	C	10.7(60)	30.7(60)	
			None	A	9.7(10)	16.7(20)	22.7(20)
1987	8.5	5.6	15.6-28.9	B	16.7(40)	11.8(40)	4.9(40)
			» - »	C	6.8(60)		
CARROT/ONION							
			None	A	25.6(20)	3.7(20)	14.7(20)
						21.7(20)	28.7(20)
1986	21.5	10.6	27.6-30.9	B	9.7(40)	25.7(40)	19.8(40)
			» - »	C	18.7(60)	19.8(60)	
			None	A	10.7(10)	16.7(20)	22.7(20)
1987	22.5	9.6	2.7-29.9	B	29.7(40)	31.8(30)	
			» - »	C	12.8(50)		

1) Refers to estimated seepage in period of high rainfall.

2500» open surface evaporimeter, which at Kise has been found to give approximately 90% of the values calculated according to Penman (1963), and to give close agreement with a short-grass lysimeter (see appendix II).

Temperatures were close to average except for June 1986, which was warmer than average, and June 1987 which was appreciably colder than average. In the latter month potential evaporation was only half of the long-term mean, and rainfall was almost three times as great. August was somewhat colder and considerably wetter than average in all three years.

Dates of sheltering and irrigation timing and quantities are given in Table 2. The sheltering periods employed in 1985 were somewhat shorter than those employed in 1986 and 1987. This was due to the inclusion of a later sheltering period in the initial year, which was subsequently abandoned because the desired deficits were not reached. The sheltering period for cereals was extended in 1987, due to flooding of the plots during extreme rainfall conditions in mid-June. The extent of this seepage was estimated from neutron-probe readings.

Deficits of 60 mm were reached twice on treatment C for all crops in 1986, but only once in the other two years. Similarly, deficits of 40 mm were reached 3-4 times on treatment B in 1986, but usually only twice otherwise.

The total amounts of irrigation plus rainfall for the whole season (Table 3) were comparable in most cases for treatments B and C, and varied between 50 and 95% of those on treatment A. Little information is gained, however, from the latter comparison, since drainage losses occurred on treatment A due to uneven rainfall distribution.

Soil description

Samples were taken from eight soil profiles arranged within a 60x20 m grid. Results of soil physical analyses are given in Table 4. The soil is classified as an imperfectly drained brown earth (gleyed melanic brunisol, Canada Dept. Agric.) derived from morainic till with a moderately high stone and boulder content. Topsoil depth was on average 28 cm and drain depth was approx. 90-100 cm.

The humus-rich, medium sandy loam topsoil had a high porosity, good aeration properties and moderately high water-holding capacity between pF 2 and pF 4.2. Silt content increased with depth, and the subsoil was characterized by about 40% lower porosity and water holding capacity. Air capacity declined to below 10% at 40 cm. The bulk density of the subsoil was genearally greater than 1.8 t m^{-3}. Such values are thought to restrict root growth (Anderson 1986). Some plant roots (10-25 per 100 cm) were observed at 30-40 cm depth, a few (1-10

Table 3. Total amounts of irrigation plus rainfall (mm) from May to September on treatment A, and relative amounts (%) for the other treatments

		Treatment			
		A (mm)	B (%)	C(%)	D(%)
CEREALS	1985	464	87	92	94
	1986	366	94	94	73
	1987	549	61	64	91
POTATO	1985	451	81	80	96
	1986	366	62	51	73
	1987	549	58	47	91
CARROT/ONION	1986	366	52	52	73
	1987	549	58	55	91

Table 4. Soil physical properties at the trial site. Means and standard deviations (in parentheses) of eight profiles at the trial site

Depth	Textural class	Sand %	Silt %	Clay %	Gravel %	Ignition loss %	Bulk density t m^{-3}
0-10cm	Sandy loam	54	33	13	19	9,1	1.22
		(3)	(2)	(3)	(4)	(1.1)	(0.04)
10-20cm	» »	55	31	14	20	8.9	1.23
		(3)	(2)	(3)	(4)	(0.8)	(0.07)
20-30cm	» »	55	32	13	15	7.2	1.37
		(4)	(2)	(3)	(2)	(1.8)	(0.11)
30-40cm	» »	62	28	10	17	2.2	1.76
		(9)	(7)	(6)	(14)	(0.6)	(0.05)
40-50cm	Sandy silt loam	49	39	12	14	2.0	1.85
		(9)	(8)	(4)	(6)	(0.8)	(0.08)
50-60cm	» » »	45	42	13	13	2.0	1.85
		(15)	(8)	(7)	(6)	(0.7)	(0.10)

Depth	Total porosity %	Air capeacity[1] %	Air permeability[1] µm^2	Water retention % pF2-3	pF3-4.2	pF2-4.2	>pF4.2
0-10cm	53.6	19.6	10.9	7.1	18.2	25.3	8.7
	(1.5)	(3.2)	(4.6)	(0.8)	(1.6)	(2.1)	(0.9)
10-20cm	53.1	19.9	11.1	7.2	17.7	24.8	8.4
	(2.3)	(3.0)	(3.8)	(0.7)	(1.3)	(1.7)	(0.7)
20-30cm	47.8	13.2	7.7	7.7	17.0	24.7	9.9
	(4.0)	(2.6)	(2.8)	(1.4)	(2.5)	(3.7)	(1.3)
30-40cm	34.2	12.0	10.1	4.5	9.8	14.3	7.9
	(1.9)	(5.2)	(13.5)	(1.1)	(1.8)	(2.3)	(3.4)
40-50cm	31.1	8.8	4.1	3.1	11.1	14.2	8.1
	(2.8)	(2.5)	(2.8)	(0.8)	(2.8)	(3.1)	(1.7)
50-60cm	31.1	7.3	2.5	3.3	11.6	14.9	8.9
	(3.7)	(2.4)	(2.4)	(1.3)	(3.4)	(3.7)	(3.2)

[1] Measured at pF 2.

per 100 cm) at 40-60 cm and none at all at depths of more than 60 cm.

Available water capacity

The water-holding capacity between pF 2 and pF 4.2 was 75 mm at 0-30 cm depth and 43 mm at 30-60 cm depth. Field capacity (FC) was found to equate well with pF 2 values in a study on a similar soil (Fig. 1), where samples were taken at 0-50 cm depth from bare soil plots which had been covered with plastic sheeting following irrigation to saturation.

Madsen and Platou (1983) found from simulation studies that pF 4.2 gave a suitable estimate of wilting point (WP) at root densities above 1 cm cm^{-3}, but that at a root density of 0.1 cm cm^{-3}, wilting point was closer to pF 3. This was later confirmed in field studies by Andersen (1986), who found that plants utilize on average only 22% of the theoretically available water (that held between pF 2 and pF 4.2) at 0.1 cm cm^{-3} root density. This corresponds closely with the proportion of water held between pF 2 and pF 3, both at 40-60 cm depth in the present study and in morainic loams generally (Riley 1979).

No measurements of root length were made in this study, but Samuelsen (1986) found that the designation «few

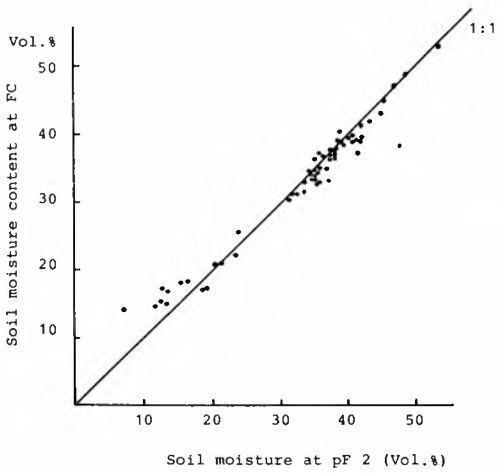


Figure 1. Comparison of moisture contents of cylinder samples equilibrated at pF 2 in the laboratory with values measured in the field at assumed field capacity. Plots were irrigated and covered with plastic sheeting for several weeks prior to sampling. Samples were taken at depths from 10 to 60 cm on morainic loam soil

roots» corresponded with a root density of approx. 0.1 cm cm^{-3}. On the basis of such reasoning, the available water capacity (AWC) was assumed here to be 95 mm for the rooting zone 0-60 cm, comprising 75 mm from 0-30 cm, 14 mm from 30-40 cm and 6 mm from 40-60 cm.

Rooting depth of individual crops

Use was made of ^{32}P as a radiotracer to estimate root growth rates and final root distribution of the crops grown. Placements of 2.1 g P (ca. 180 µCi activity) were made by augering at 10 cm depth intervals from 5 to 45 cm on adjacent plots of a nearby trial in 1984 and 1985, and placements of 0.9 g P (ca. 90 µCi activity) were made at 15 cm intervals from 10 to 75 cm in 1987 in the present trial. Three replicates were used in all cases. Root growth rates were assessed from activity readings made by holding a GM-counter above the plants closest to the point of ^{32}P-application. This method was unsuccessful in onions and at the lower application rate.

Roots were judged to have reached a particular depth when two of three replicates showed clear signs of uptake. Plants were harvested for analysis in August. Results of this analysis were expressed as percentages of the values at the depth with greatest activity.

RESULTS AND DISCUSSION

Validation of parameters in the soil moisture model

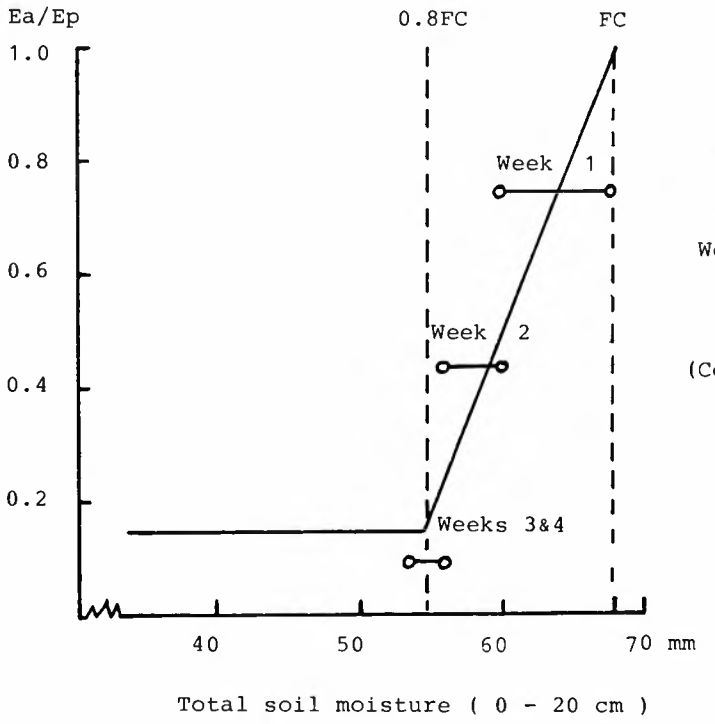
Evaporation from bare soil

Evaporation from soil with no plant cover declines in the model to 15% of the potential rate (Ep) when 20% of the water held at field capacity has been depleted in the upper 20 cm of soil (i.e. 14 mm in the present case). This assumption was found to be appropriate when the evaporation from bare soil was monitored on sheltered plots over four weeks in 1987, following heavy rainfall (Fig. 2).

Further confirmation was obtained from comparisons of model predictions with soil moisture measurements made in spring over four years in a previous study at the same site (Ekeberg 1985). The effect of varying the depletion constant by 4 mm above and below 14 mm is shown in Fig. 3. Neither choice gave consistently closer agreement with the measured moisture values, and the intermediate value of 14 mm was therefore accepted.

Development of plant cover

The use of standard values for the increase and decline of green plant cover in spring and autumn was studied over three years in oats (Table 5). Data for actual evapotranspiration (Ea) were derived from changes in soil moisture on sheltered plots which were irrigated to field capacity at the start of each period. Good agreement was found at all growth stages when the leaf area index (LAI) was assumed to reach 2.8 three weeks after crop emergence and to decline again to zero three weeks later than the



	Evaporation (mm)		
		Meas.	Calc.
	Ep	Ea	Ea
Week 1	10.5	7.8	8.1
Week 2	8.1	4.0	3.6
Weeks 3&4	23.5	2.2	4.4

(Constant = 0.8FC = 14 mm)

Figure 2. Relative evaporation from bare soil according to the model of Kristensen & Jensen (1975), illustrated for soil which was sheltered from rainfall over four weeks at Kise Research Station. (o——o = measured values, EP = potential evaporation, Ea = actual evaporation, FC = field capacity)

Table 5. Comparison of measured and calculated ratios of actual to potential evapotranspiration (Ea/Ep) at different growth stages of oats. Calculations made with model of Kristensen and Jensen (1975) using standard values for green plant cover

Growth stage	Year	Dates	Ep,mm	Ea/Ep Measured	Ea/Ep Calculated
Sowing to tillering	1981	7.5-2.6	58	0.48	0.48
	1982	10.5-2.6	42	0.58	0.56
	1983	25.5-7.6	17	0.75	0.78
Tillering and stem elongation	1981	3.6-22.6	43	0.72	0.92
	1982	3.6-23.6	76	0.70	0.79
	1983	8.6-27.6	62	0.78	0.83
Booting and inflorescence	1981	23.6-13.7	40	0.91	0.90
	1982	24.6-14.7	53	0.74	0.86
	1983	28.6-19.7	63	0.64	0.62
Anthesis and milk development	1981	14.7-3.8	47	0.86	0.90
	1982	-	-	nd	nd
	1983	20.7-9.8	55	0.98	0.93
Dough development and ripening	1981	4.8-24.8	45	0.60	0.63
	1982	5.8-12.8	35	0.75	0.71
	1983	10.8-30.8	55	0.41	0.52

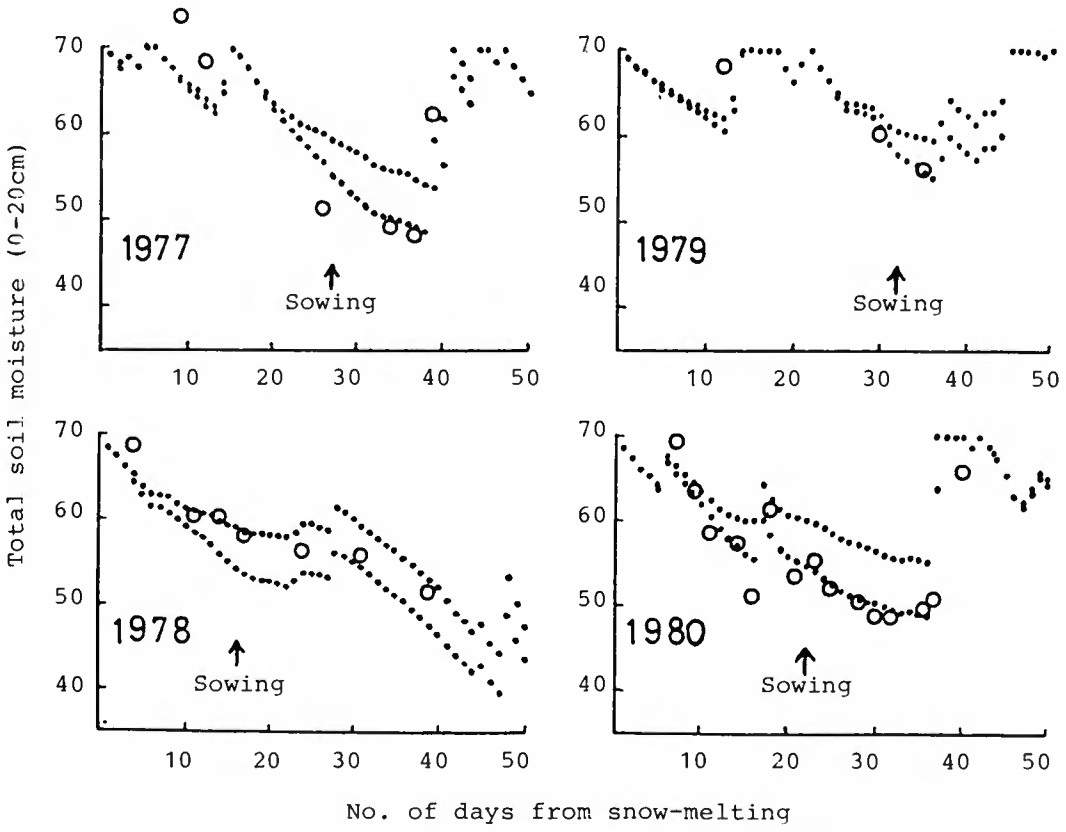


Figure 3. Soil moisture content (mm) at 0-20 cm depth of a moranic loam soil in spring. Dots (···) represent values calculated according to the model of Kristensen & Jensen (1975), using constant values of 10 and 18 mm (upper and lower lines respectively). Circles (o) represent values measured at Kise

milk development stage.

Measurements made in all years of the present trial (Fig. 4) showed that LAI of both cereals and potatoes reached the value required for full evapotranspiration (2.8) approximately as predicted. This value corresponded in potatoes with the time at which foliage just met between rows. The latter stage was reached in mid to late July in the carrot and onion crops. Confirmatory measurements of LAI were made in onions.

Rooting depth and root zone capacity

The time taken for uptake of ^{32}P from different depths is shown in Fig. 5. Roots of both barley and potato appeared to penetrate to 45 cm within three to four weeks of crop emergence, indicating similar growth rates (1.5-2 cm/day) to those cited by Aslyng and Hansen (1982). Water uptake was therefore probably possible from most of the stipulated root zone (0-60 cm) at the growth stages when irrigation was given to these crops in the present study. Little activity was found in carrots until about 4 weeks after emergence, after which the growth rate was similar to that of the other crops.

The distribution of ^{32}P in plants harvested in August is shown in Fig. 6. The results for barley indicated a high root density at shallow depth (< 15 cm), and a low or moderate density at all other depths. Haahr (1968) found a similar

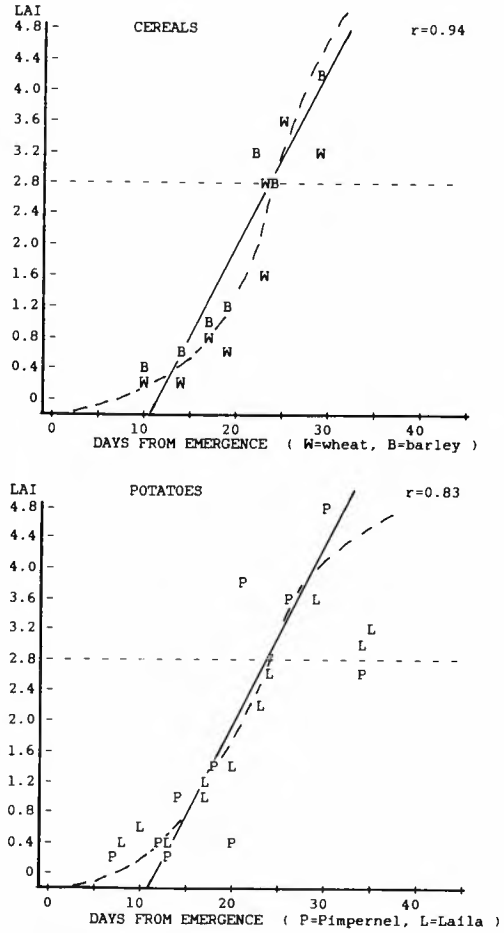


Figure 4. Development of leaf area index (LAI) in cereals and potatoes. Pooled data for 1985-1987

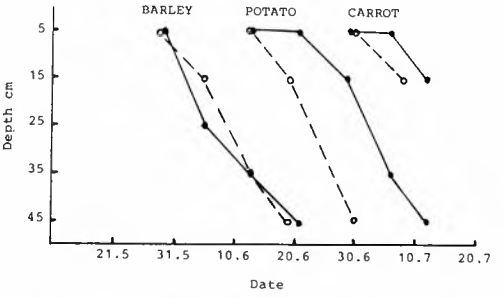


Figure 5. Root penetration rate of various crops on a sandy loam soil, assessed by ^{32}P uptake from different depths. (● = 1984 sowing/planting 11.5, o = 1985 sowing/planting 21.5

concentration of cereal roots near the soil surface. There was a consistently high uptake of ^{32}P from intermediate depths in potatoes (30-40 cm from the ridge-top, or 20-30 cm from the mean surface level). Root activity of both onions and carrots appeared to be concentrated mostly in the topsoil. The former is known to be a shallow-rooted crop (Aslyng and Hansen 1982), but carrots may be expected to have exhibited deeper rooting later in the season.

Further information on the distribution of roots was obtained from changes in soil moisture profiles during periods without irrigation and with little or no rainfall. Such data are presented in Fig. 7 for all crops in two seasons with high (1986) and low (1987) evaporative demand respectively. Little change was found in moisture profiles towards the end of these periods, indicating that water uptake was severely limited. Furthermore, signs of drought stress were clearly visible in all crops.

Fig. 8 shows the maximum percentage depletion of water held at different depths between «field capacity» (the highest field measured value for each profile) and the laboratory-determined values at pF 4.2. The former values were about 15% lower than the values measured in the laboratory at pF 2. This is probably due to the fact that the neutron-probe readings are affected by the high stone and boulder content of this soil, whereas this is not taken into account in the pF-analysis. The field-measured values were judged to be most realistic for the present purpose.

Cereals utilized about 80-100% of the moisture available in the topsoil, whilst the other crops used about 70-90%. At 30-50 cm depth the figure was about 50% for all crops, declining to around 20% at 50-70 cm and to zero below 70 cm. Such figures accord well with the values found by Andersen (1986) for Danish soils at different root densities (86% at 10 cm cm^{-3}, 43% at 1 cm cm^{-3}, 22% at 0.1 cm cm^{-3} and 11% at 0.01 cm cm^{-3}), and support the choice of 95 mm as

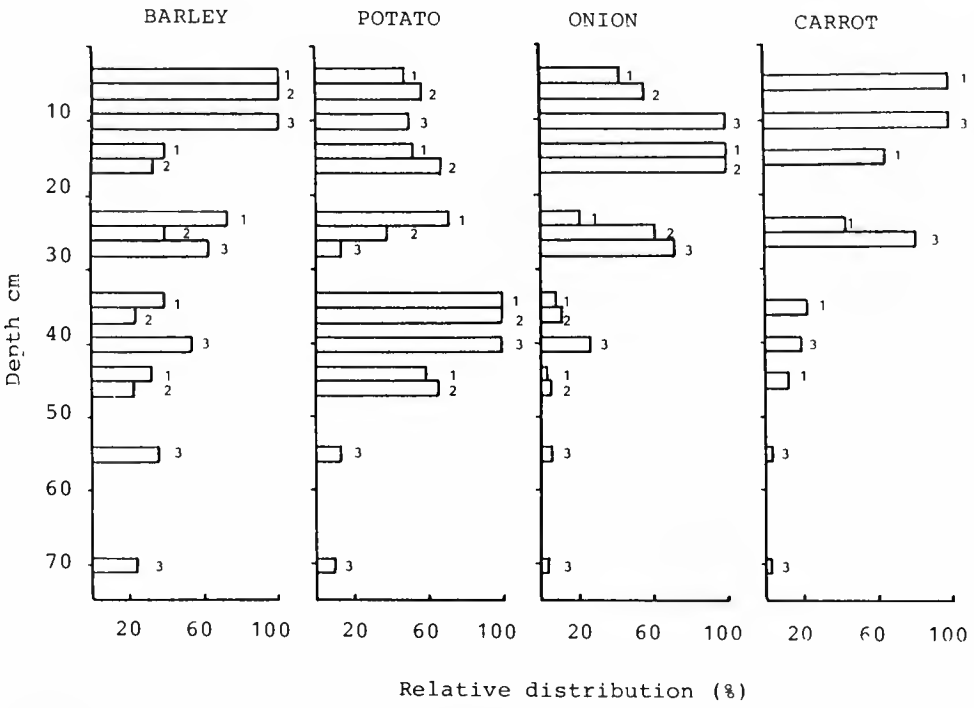


Figure 6. Relative distribution of ^{32}P taken up from different depths of a morainic loam soil at Kise. Samples were taken in August. (1 = 1984, 2 = 1985, 3 = 1987)

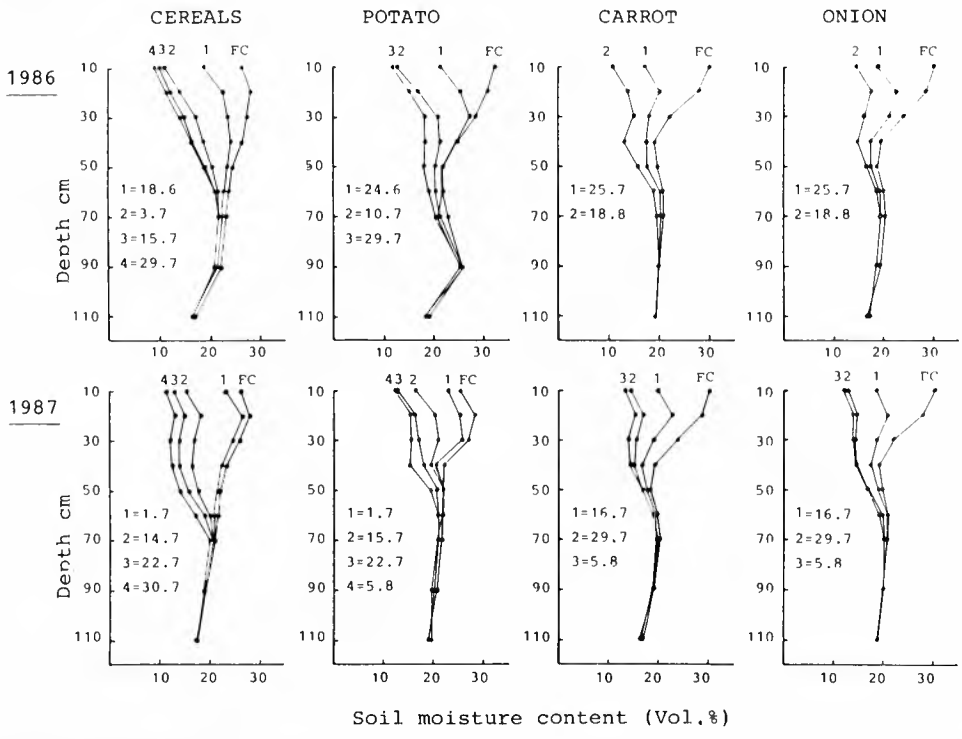


Figure 7. Soil moisture profiles for different crops in periods with sheltering or little rainfall. FC = maximum measured values in field plots. All data are means of two profiles

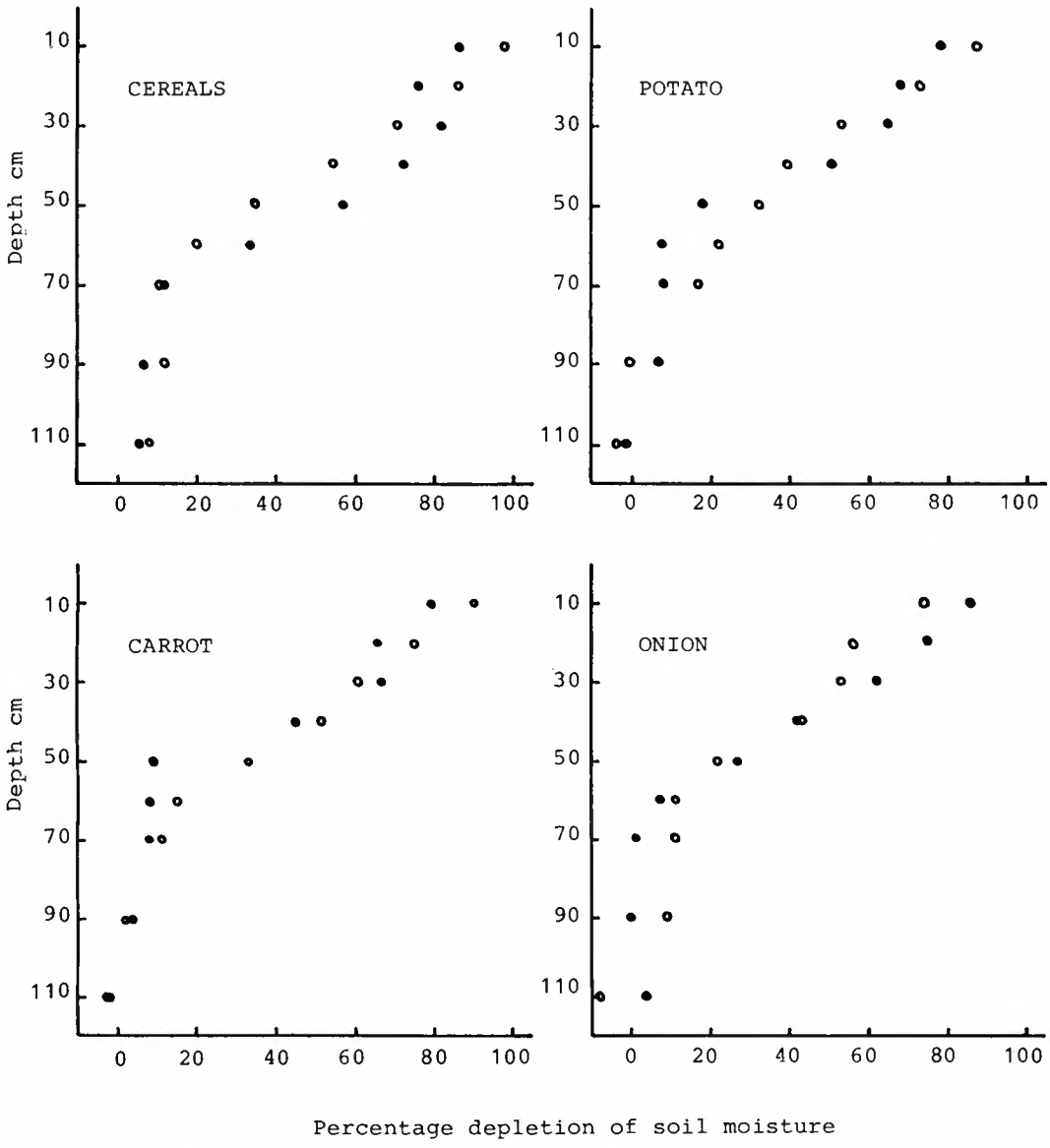


Figure 8. Percentage depletion of soil moisture held between field capacity and pF 4.2, at various depths during drought periods. (o = 1986m ● = 1987)

an appropriate value for the available water capacity (AWC) of the root zone in the present study.

Evapotranspiration in relation to soil moisture deficit

Estimates of actual evapotranspiration (Ea) were derived for cereals and potatoes from measurements of soil moisture changes during selected 1-3 week periods with full plant cover (LAI >2.8) in June/July. Data were excluded in cases where drainage losses were suspected. Ratios of Ea to Ep are plotted in Fig. 9 against the mean of soil moisture deficits measured at the start and end of each period.

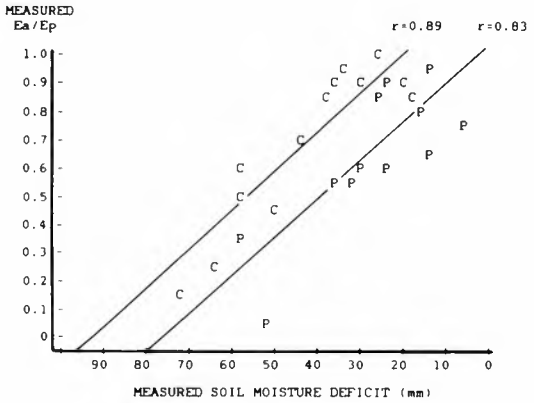


Figure 9. The measured ratio of actual to potential evapotranspiration (Ea/Ep) relative to the mean moisture deficit measured in selected periods with full plant cover in June/July. (C = cereals, P = potatoes)

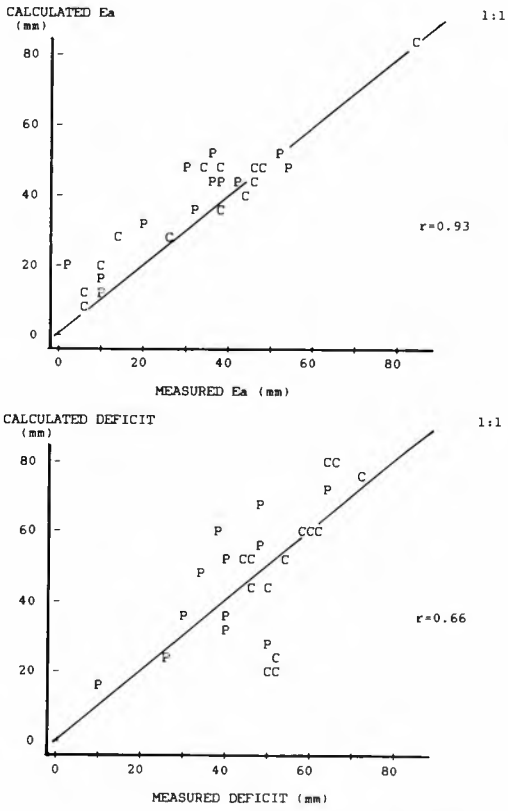


Figure 10. Calculated values for actual evapotranspiration (above), and soil moisture deficit (below), in relation to values measured in selected periods with full plant cover in June/July (C = cereals, P = potatoes)

Both crops showed a linear decline in Ea/Ep rates as deficits increased. Evapotranspiration would appear, by extrapolation, to cease at a deficit of about 80-100 mm, in agreement with the proposed AWC-value. The data suggest a somewhat greater sensitivity of potatoes than of cereals to restricted soil moisture. This is in accordance with the suggestion by Slabbers (1980) that the former crop exhibits a higher critical leaf potential than cereals. Variation of the relevant constant in the model has been found, however, to have only a minor influence on calculated Ea-rates under the climatic conditions at Kise (Riley 1981).

The estimated Ea-values were slightly lower than those calculated using the water budget model (Fig.10, above). The reason for this is not clear.The validity of the model calculations is supported, however, by the fact that moisture deficits measured at the end of each period showed no consistent deviation from predicted values (Fig. 10, below).

Crop yields and quality

Cereals (Table 6)

Grain yields were corrected in two cases where measured values showed marked divergence from the relationship between straw and grain yield (trt. A for both barley and wheat in 1985, and trt. B and C for wheat in 1987). Grain development had been hampered in both cases due to lodging followed by cold, wet weather. Such lodging was thought to be caused by the high impact-pressure of the small-plot irrigation equipment used.

Yields of both grain and straw were greatest in all years at the highest irrigation frequency (trt. A). Straw yields showed a greater response to water supply than grain. Dragland (1978) reported a similar effect for wheat but not for barley, whilst Ekeberg (1982) and

Table 6. Cereal yields and quality

		Irrigation interval (deficit)				
		20 mm	40 mm	60 mm	Non-irrig. (rain-fed)	s.e. of mean
STRAW	1985	5.96	4.54	4.17	5.63	
(t ha^{-1})	1986	4.93	4.09	3.22	3.08	0.13
	1987	6.61	5.75	5.55	6.46	
	Mean	5.83	4.79	4.31	5.06	0.07
	Barley	4.66	4.00	3.55	4.23	
	Wheat	7.00	5.58	5.07	5.89	
GRAIN	1985	5.17	4.58	4.45	5.01	
(t ha^{-1})	1986	4.94	4.37	3.84	3.74	0.14
	1987	4.35	4.00	3.97	4.42	
	Mean	4.82	4.32	4.09	4.39	0.06
	Barley	4.73	4.48	4.21	4.42	
	Wheat	4.91	4.16	3.97	4.36	
GRAIN QUALITY						
N % in	Barley	1.8	1.9	2.0	1.7	
grain	Wheat	1.9	2.1	2.1	2.0	0.05
Weight	Barley	38.6	38.6	38.5	39.2	
per 1000 grain (g)	Wheat	37.4	35.9	36.3	38.0	0.7
Bulk weight	Barley	70.2	69.8	70.2	71.1	
(kg hl^{-1})	Wheat	81.3	80.0	80.4	81.3	0.3
Falling number (s)	Wheat	244	214	195	213	14

Jørgensen (1980) found that irrigation increased grain yields by as much as or more than straw yields. Results presented by Myhr & Rognerud (1974) from a 13-year trial in barley showed considerable variation in the relative responses of grain and straw.

Withholding irrigation until a deficit of 60 mm (trt. C) resulted in lower grain yield than irrigation at 40 mm deficit (trt. B) in only one year. Barley yields showed slightly less response to irrigation frequency than wheat yields. The latter trend has been found in previous irrigation trials in Norway (Dragland 1978, Hauge et al. 1981).

There was a slight increase in grain N-concentration when irrigation was postponed (trt. B & C), presumably due to the lower yield levels. No significant effect was found in the concentration of P and K, nor in quality properties related to grain size and density. There was, however, a tendency towards higher values of falling number as irrigation frequency increased. A beneficial effect of irrigation on this quality parameter has frequently been found in earlier studies (Dragland 1978, Ekeberg 1982, Hauge et al. 1981), and is thought to be related to a reduction in late tillering which leads to uneven ripening.

Potatoes (Table 7)

The overall trend in yield effects was of the same magnitude as that in cereals, and responses were similar for total tuber yield, ware yield and DM yield. The treatment with most frequent irrigation gave a lower yield in 1985 than the non-irrigated treatment. It is unlikely that this can be attributed to a difference in nutrient leaching, since the former treatment received only 20 mm

Table 7. Potato yields and quality

		Irrigation interval (deficit)				
		20 mm	40 mm	60 mm	Non-irrig. (rain-fed)	s.e.of mean
TOTAL TUBER YIELD ($t\ ha^{-1}$)	1985	36.4	37.8	36.5	38.1	
	1986	38.0	33.6	30.5	28.7	1.3
	1987	39.1	37.9	33.9	37.4	
	Mean	37.9	36.4	33.6	34.7	1.0
	cv. 'Laila'	42.3	39.8	37.5	39.4	
	cv. 'Pimpernel'	33.4	33.0	29.7	30.1	
WARE YIELD >45 mm ($t\ ha^{-1}$)						0.8
	cv. 'Laila'	33.7	31.6	28.6	31.1	
	cv. 'Pimpernel'	25.4	24.1	20.7	22.3	
DM PERCENTAGE						0.6
	cv. 'Laila'	20.9	21.3	21.8	20.6	
	cv. 'Pimpernel'	25.7	25.5	26.1	24.4	
DM YIELD						0.3
	cv. 'Laila'	8.80	8.38	8.00	8.14	
	cv. 'Pimpernel'	8.56	8.39	7.70	7.38	
						0.22
NO. of TUBERS PER PLANT						
	<35 mm	1.6	2.2	2.3	1.8	0.10
	35-45 mm	3.0	3.1	3.2	2.9	0.09
	>45 mm	4.8	4.5	4.3	4.4	0.16
MINERAL CONTENTS (% of DM)						
	N	1.15	1.19	1.22	1.23	0.03
	P	0.26	0.25	0.23	0.26	0.01
	K	1.88	1.92	1.93	2.00	0.03

irrigation in that year. Postponing irrigation until 60 mm deficit reduced yields in all three years compared with irrigation at 40 mm deficit.

The effects of irrigation were similar in both varieties. 'Laila' had considerably higher tuber yields than 'Pimpernel', but lower DM percentage, as is normal for this variety. The total DM yields were therefore similar for both varieties.

Dry matter percentages were slightly higher in all irrigated treatments than in the non-irrigated treatment. The effect was most marked in the extremely dry conditions of 1986, but was also present in the wet year of 1987. It is well documented that drought in early or mid-season leads to lower DM percentages in potatoes (Dragland 1978, 1985, Jørgensen 1980, Myhr 1970). However, in the present trial, delaying irrigation until 40 or 60 mm deficit caused no reduction in tuber DM relative to irrigation at more frequent intervals.

The total number of tubers per plant was 50% greater in the cold, wet year of 1987 than in other years, but showed no consistent effect of irrigation treatment. The distribution between size groupings varied between years, but there was an overall trend towards fewer non-saleable tubers (<35 mm) and more ware tubers (>45 mm) in the treatment with most frequent irrigation relative to irrigation at 40 and 60 mm deficit.

Tuber concentrations of both N and K were lower in irrigated treatments than in the non-irrigated treatment, and were lowest in the treatment with most frequent irrigation. Similar findings are reported by Ekeberg (1986), Jørgensen (1984a) and Linnér (1984). In the case of

nitrogen this is probably a dilution effect related to yield level, whilst for potassium it may be attributed to more rapid maturing. The ratio of N to K, which is of importance in connection with tuber discolouration (Enge and Bærug 1971), was not affected by irrigation. No significant effect was found in the case of phosphorus.

Carrots and onions (Table 8)
In both years the yields of carrot and onion showed greater responses to irrigation frequency than either cereals or potatoes. Postponing irrigation until deficits reached 60 mm reduced yields of the latter by about 15% compared with the most frequent irrigation treatment, whilst the reduction was 28% in onion and 33% in carrot, both for total and saleable yields.

Postponement of irrigation gave a reduction in the number of saleable carrots, mainly due to inadequate root size. The treatment with most frequent irrigation gave the highest proportions of split roots, fanged roots and roots that were too large for the retail market. This was nevertheless more than compensated for by higher yield levels.

The frequent-irrigation and rain-fed treatments in onion gave greater weight loss on drying. This may simply have been a consequence of the sheltering of the other two treatments. The latter treatments caused a slight increase in percentage bolting, but at a low level in all cases. No significant effects of irrigation were found on skin quality or neck thickness.

Table 8. Carrot and onion yields and quality

		Irrigation interval (deficit)				
		20 mm	40 mm	60 mm	Non-irrig. (rain-fed)	s.e. of mean
CARROT						
Total yield	1986	78.2	61.6	58.7	60.5	
(t ha^{-1}	1987	60.3	41.3	34.0	53.3	
	Mean	69.3	51.5	46.4	56.9	0.9
Saleable yield (Factory, >2 cm diam.)		60.6	45.6	40.5	49.3	1.1
Saleable yield (Retail, 2-5 cm diam.)		47.7	35.0	31.7	38.4	1.4
Yield, <2 cm diam.		2.4	2.8	3.0	3.9	0.3
Yield, >5 cm diam.		2.3	0.4	0.1	0.4	0.2
Yield, split roots		1.6	0.8	0.6	0.9	0.2
Yield, fanged roots		4.7	2.2	2.2	2.8	0.2
No. saleable roots m^{-2}		77	66	62	75	1
Total no. roots m^{-2}		108	100	99	117	3
ONION						
Total yield	1986	45.6	35.2	33.1	30.2	
(t ha^{-1}) after	1987	53.5	43.0	38.1	45.4	
drying[1])	Mean	49.5	39.1	35.6	37.8	1.3
Yield of bulbs >3 cm		48.8	39.0	35.0	37.4	1.3
Weight loss on drying (%)		11.5	8.8	8.5	12.9	0.5
Percentage bolting		1.3	1.7	2.6	1.7	0.3

[1]) Drying at 25-30°C over 3-4 weeks.

Relation of yields to evapotranspiration

Models of yield responses to evapotranspiration enable the comparison of different irrigation strategies over longer periods than those for which trial data are available. This is especially relevant in the present case, where drought conditions were induced by sheltering. Regression equations of the type used by Gregersen and Olesen (1983) were derived for each crop.

$$Y/Ym = a + b_1 \cdot (Ea/Ep)_1 + \cdots b_n \cdot (Ea/Ep)_n$$

where:

Y	= yield of individual treatment
Ym	= yield of treatment with most frequent irrigation
$b_{1,n}$	= sensitivity factor of first, last growth stage
$(Ea/Ep)_{1,n}$	= calculated relative evapotranspiration rate during first, last growth stage.

The use of relative yield data removes between-year variation caused by factors other than water supply. Growth stages were defined according to the standard LAI-values used in the moisture budget model. Various combinations and period lengths were compared in each case. The best-fit equations, in which all included terms were significant with at least 95% probability, are given in Table 9. Observed and predicted values are shown in Fig. 11.

A joint equation for both cereals accounted for a greater proportion of variance than individual equations for barley and wheat. Greatest sensitivity to drought was found at 4 to 6 weeks from germination (tillering to heading). This concurs with the findings of Andersen (1987), Day et al. (1978), Dragland (1979), Jørgensen (1979) and Mogensen (1980). A lower sensitivity was found for the 7 to 10 week period (heading to anthesis), but there was no effect at other growth stages.

Similar equations were found for both DM and ware potato yields. Separate equations for cv. 'Laila' and cv. 'Pimpernel' differed only in that early drought (1 to 3 weeks from emergence) appeared to be harmful to the former variety but beneficial to the latter. Neither trend was significant, although both support the findings of Dragland (1985). For the present purpose, a joint equation with a single sensitivity factor for the whole of the period with full leaf cover (4 to 14 weeks) was considered

Table 9. Regression equations of relative yields (Y/Ym)[1] and relative evapotranspiration (Ea/Ep) at different growth stages

Crop	Equation	R^2
CEREALS (barley/wheat)		
	$Y/Ym = 148 \times (Ea/Ep)_1 + 15 \times (Ea/Ep)_2 - 62.3$ 1 = 4-6 weeks and 2 = 7-10 weeks from emergence	0.92
POTATO (ware)		
	$Y/Ym = 174 \times (Ea/Ep)_1 - 70.0$ 1 = 4-14 weeks from emergence	0.81
CARROT (factory)		
	$Y/Ym = -32 \times (Ea/Ep)_1 + 123 \times (Ea/Ep)_2 + 170 \times (Ea/Ep)_3 - 150.1$ 1 = 1-4 weeks 2 = 5-8 weeks and 3 = 9-16 weeks from emergence	0.97
ONION (>3 cm)		
	$Y/Ym = 151 \times (Ea/Ep)_1 + 18 \times (Ea/Ep)_2 - 51.9$ 1 = 7-10 weeks and 2 = 11-18 weeks from planting	0.95

[1])Relative yields are expressed as percentages.

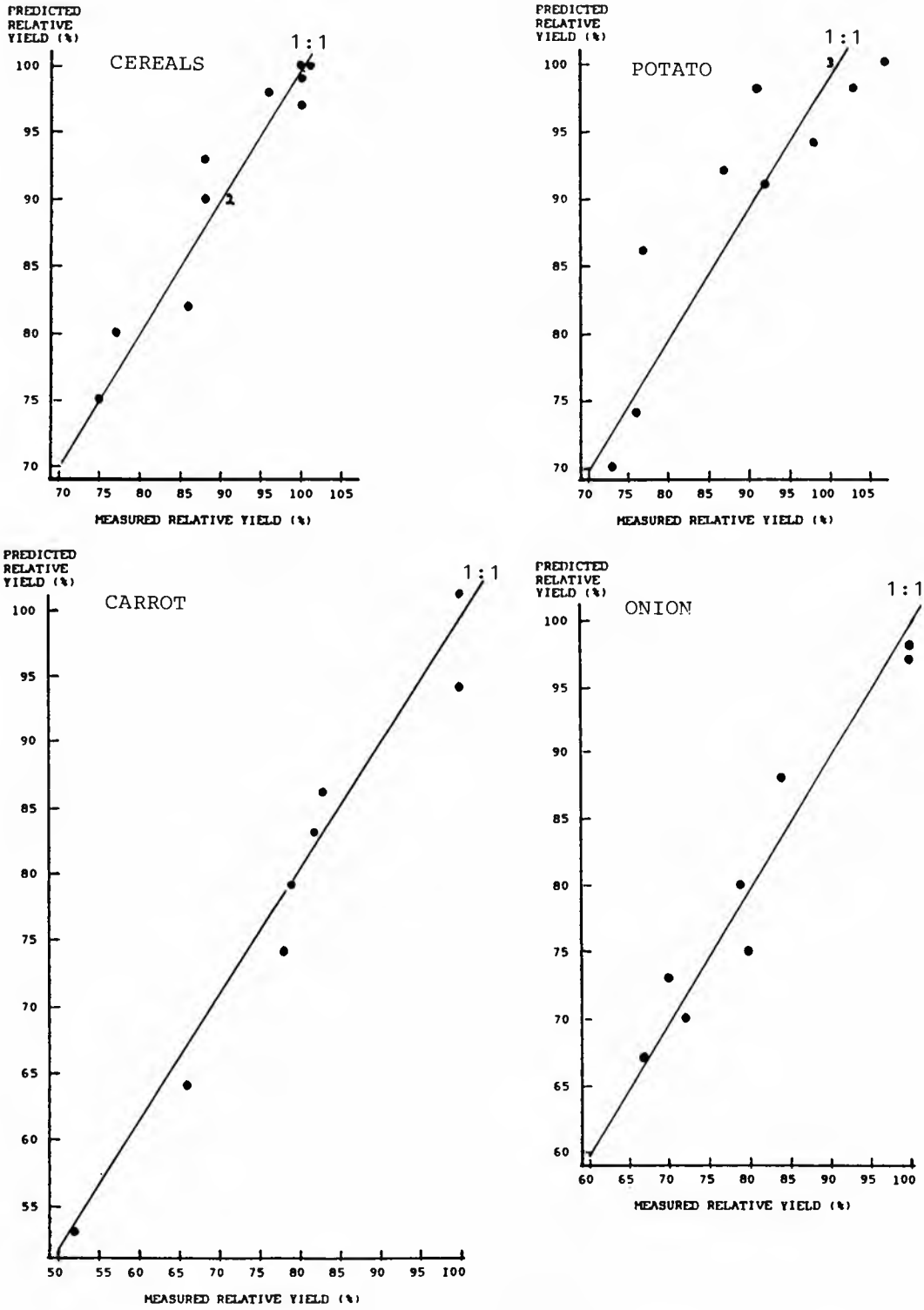


Figure 11. Relative yields predicted from regression on relative evapotranspiration at various stages of growth, compared with relative yields measured in field trials

most appropriate.

Carrots were sensitive to drought both in mid-season (5 to 8 weeks from emergence) and up to harvest (9 to 16 weeks). A beneficial effect of early-season drought (1 to 4 weeks) accounted for 12% of the variance in this crop. Dragland (1978a) found that yields increased by 15% when carrots were sheltered at this period. The reason may lie in higher soil temperature or reduced nutrient leaching.

Onions showed no effect of early drought (3 to 6 weeks from planting), but marked sensitivity in mid-season (7 to 10 weeks), which coincides with the period of maximum leaf growth. The differentiation between foliage and fleshy (bulb) leaves is controlled primarilly by daylength, but temperature and moisture conditions are also important (Balvoll 1981). It is thought that the negative effect of drought at this stage is due to premature bulb formation (Dragland 1974). A lower sensitivity was found later in the season.

Prediction of yield responses to irrigation

Calculations were made of relative yields over twenty-five years, using the regression equations presented above, for strategies with irrigation at the following soil moisture deficits (% of AWC) in sensitive growth stages:

Strategy	First sensitive growth stage	Second sensitive growth stage
I	25%	25%
II	25%	50%
III	50%	50%
IV	50%	75%
V	75%	75%

All calculations were made for available water capacities of 70, 90 and 110 mm.

Sensitive growth stages for cereals, carrot and onion were the same as those given in Table 9. For potato, the 4 to 14 week period from emergence was arbitrarily divided into 5 and 6 week periods.

The date of emergence of cereals was set at 15th May in all years, and that of potatoes and carrots at 5th June. Onion leaf growth was assumed to start on the former date. These dates correspond with the normal times for sowing/planting of these crops in the district near Lake Mjøsa (early May for cereals, potatoes and onions, and around 21st May for carrots). Irrigation was thus given whenever the specified deficits occurred in the periods 6th June to 24th July in cereals, 27th June to 11th Sept. in potatoes, 4th June to 30th Sept. in carrots and 13th June to 11th Sept. in onions.

Daily evaporation and precipitation data for the period 1963-1987 from Kise Research Station were used as input for the calculations. Moisture budgets were calculated from the day after snow-melting in individual years (usually around 15th April), when the soil was assumed to be at field capacity.

In cases where evaporimeter data were lacking, values were estimated from relationships of pan evaporation with either Penman's equation or with air temperature. Such cases occurred mostly in April or early May, and had little influence on calculated irrigation requirements.

Output included average data for each strategy of yield levels, actual evapotranspiration, irrigation quantities and losses of water to drainage. The frequency distributions of various groupings of percentage yields without irrigation were calculated, relative to the strategy with most frequent irrigation. Relative yields are also given for years with low (6-15%), moderate (16-25%) and high (>25%) yield reduction in the absence of irrigation. Results for soil with moderately high available water capacity (90mm) are given in Tables 10-12.

The long-term average maximum responses to irrigation on such soil were around 28% in potato and carrot, and around 18% in cereals and onion (Table 10). These figures reflect the length of

Table 10. Predicted yield levels, actual evapotranspiration (Ea), irrigation amounts and losses to drainage for various irrigation strategies. Figures are means for 1963-1987 at Kise Research Station. Calculations are for AWC = 90 mm

	Strategy[1]	CEREALS	POTATO	CARROT	ONION
Relative yield %	I	100.0	100.0	100.0	100.0
	II	99.8	99.3	99.0	99.8
	III	97.1	98.4	96.6	95.1
	IV	96.8	96.6	94.0	94.7
	V	90.9	94.0	90.9	89.9
	Non-irrig.	84.2	77.8	78.0	85.0
Ea (mm, May-Sept.)	I	288	291	275	296
	II	287	290	273	294
	III	285	289	272	291
	IV	281	287	270	288
	V	278	284	267	284
	Non-irrig.	260	264	256	265
Irrigation amount (mm)	I	89	105	84	110
	II	77	91	69	88
	III	67	71	62	76
	IV	49	56	51	53
	V	38	50	36	47
Drainage loss (mm, May-Sept.)	I	120	131	129	130
	II	110	121	118	112
	III	101	101	113	104
	IV	89	91	105	86
	V	82	87	91	83
	Non-irrig.	68	71	81	69

[1]) *Allowable deficits (mm)*

Strategy	First growth stage	Second growth stage
I	22.5	22.5
II	22.5	45
III	45	45
IV	45	67.5
V	67.5	67.5

the period in which each crop is most sensitive to drought.

Strategy IV, with irrigation at 45 mm deficit in the first sensitive growth stage and at 67 mm in the second, gave an average yield level within 3.5% of the maximum for cereals and potato, and within 6% for carrot and onion. Such a strategy more than halved the amount of irrigation water necessary, and reduced potential losses to drainage by about three-quarters, relative to the most intensive irrigation strategy.

Reductions of maximum potential yield in the absence of irrigation (Table 11) were less than 6% in almost half the total number of years for all crops. Reductions of more than 25% occurred in approximately one year in three in potato and carrot, one year in four in cereals and one year in five in onion.

The choice of irrigation strategy may to some extent depend upon the conditions in individual years. However, even in years with high potential yield reduction (Table 12), strategy III gave yields of within 6% of the maximum potential in cereals, potato and carrot, whilst

Table 11. Frequency distributions (% of years) of predicted yield reduction in various percentage groupings relative to irrigation whenever deficits exceed 25% of AWC (AWC = 90 mm)

	Strategy[1]	Yield reduction <6%	6-15%	16-25%	>25%
CEREALS	II	100	0	0	0
	III	84	16	0	0
	IV	76	24	0	0
	V	48	20	32	0
	Non-irrig.	44	20	12	24
POTATO	II	100	0	0	0
	III	96	4	0	0
	IV	76	24	0	0
	V	56	32	12	0
	Non-irrig.	44	24	4	28
CARROT	II	100	0	0	0
	III	80	20	0	0
	IV	56	44	8	0
	V	44	32	24	0
	Non-irrig.	44	20	8	28
ONION	II	100	0	0	0
	III	48	52	0	0
	IV	40	60	0	0
	V	40	28	20	12
	Non-irrig.	40	20	20	20

[1]) As in Table 10.

Table 12. Predicted relative yields for various irrigation strategies in years with low (6-15%), moderate (16-25%) and high (>25%) reduction in potential yield in the absence of irrigation. Calculations are for AWC = 90 mm

	Strategy[1]	CEREALS	POTATO	CARROT	ONION
Years with low (6-15%) potential yield reduction	II	99.7	99.4	99.1	99.9
	III	96.7	98.7	95.6	93.9
	IV	96.6	96.9	92.2	93.5
	V	91.2	93.5	90.5	92.0
	Non-irrig.	89.2	90.2	89.9	91.4
	No. years	5	6	5	5
Years with moderate (15-25%) potential yield reduction	II	99.8	100.0	98.8	99.8
	III	97.0	99.4	98.8	92.0
	IV	96.4	99.4	94.1	91.4
	V	84.8	93.7	94.1	83.5
	Non-irrig.	81.8	83.7	82.1	80.4
	No. years	3	1	2	5
Years with high (>25%) potential yield reduction	II	99.7	98.5	98.0	99.7
	III	94.7	96.6	94.1	90.9
	IV	94.2	92.1	89.0	90.3
	V	80.0	87.5	79.3	75.7
	Non-irrig.	55.7	33.7	37.0	55.0
	No. years	6	7	7	4

1) As in Table 10.

strategy IV gave yields within 10% of the maximum in all crops. The results for onion reflect the importance of maintaining good water supply during the period with maximum leaf growth.

Postponement of irrigation until deficits reached 75 mm regardless of growth stage (Strategy V) gave, in the driest years, yield declines of about 12% for potato and of around 20-25% for the other crops,relative to the maximum potential. This strategy nevertheless gave a 40% increase in cereal and onion yields and a doubling of potato and carrot yields in such years, relative to yields without irrigation. In years with less extreme drought, however, yield responses with such a strategy would appear unlikely to be worthwhile.

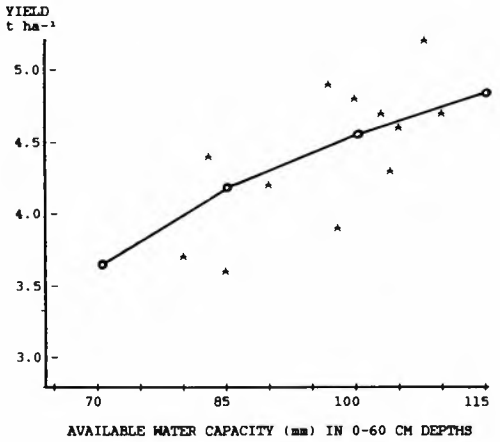


Figure 12. The effect of available water capacity in the root zone on calculated unirrigated cereal yield (O—O), assuming a potential yield of 5.5 t ha^{-1}. Data points (*) are mean yields of barley and oats, recorded on different blocks of a field trial at Kise. All data are averaged over three dry years(1982, 1983, 1986)

Responses to irrigation at different levels of AWC

Studies of morainic loam soils in southeast Norway have revealed a range of AWC-values from about 70 to 110 mm, when calculated as in the present study (Riley 1979 and unpublished data). The effect of this range of AWC on cereal yields is illustrated in Fig. 12, using data for three dry years.

Data points are the yields recorded on each block of a non-irrigated field trial adjacent to that discussed in this paper. There was a marked between-block variation in AWC in this trial. Calculated values were derived using the same regression model as before, with appropriate AWC-values and assuming a maximum potential yield of 5.5 t ha^{-1}, which is considered a realistic mean for the varieties used.

The agreement between calculated and recorded yields appeared to be satisfactory. Slight discrepancies were to be expected, both in view of the arbitrary definition of potential yield and because other factors may also have influenced the recorded yields.

Predicted long-term yield increases of all crops are given in Table 13 for two irrigation strategies (I and IV) at AWC-values of 70 and 110 mm respectively. Percentage yield responses were calculated relative to non-irrigated crops, whilst absolute responses (t ha^{-1}) were derived using assumptions of maximum potential yield made on the basis of the present and previous irrigation studies at Kise Research Station (5.5 t ha^{-1} for cereals, 35 t ha^{-1} for ware potatoes, 60 t ha^{-1} for factory carrots and 50 t ha^{-1} for onions). Frequency distributions of percentage yield reductions, relative to the maximum potential, are given in Table 14.

Responses with irrigation strategy I were approximately twice as great at an AWC-value of 70 mm as at a value of 110 mm. The difference was even greater with irrigation strategy IV, except in the case of potatoes. This crop is able to compensate for short periods of drought provided the growing season is long enough.

The frequency of years with little response to irrigation fell to about one-third at an AWC-value of 70 mm, whilst the number of years with yield reduction greater than 25% in the absence of

Table 13. Predicted yield responses, irrigation amounts and drainage losses for two irrigation strategies on soil with different available water capacity (AWC). Figures are means for 1963-1987 at Kise Research Station

	AWC	Strategy[1]	CEREALS I	CEREALS IV	POTATO I	POTATO IV	CARROT I	CARROT IV	ONION I	ONION IV
Yield response	70 mm	%	32	28	40	35	44	36	26	21
		t ha^{-1}	1.32	1.15	10.0	8.8	18.5	15.0	10.5	8.1
	110 mm	%	12	9	21	17	19	12	12	7
		t ha^{-1}	0.58	0.42	6.1	5.1	9.7	6.2	5.5	2.9
Irrigation amount (mm)	70 mm		93	58	106	74	91	51	115	71
	110 mm		80	35	95	55	76	36	102	50
Amounts lost to drainage[2] (mm)	70 mm		53	26	59	34	52	21	64	30
	110 mm		45	14	52	23	40	13	57	19

1) I = Irrigation at 17.5 or 27.5 mm deficit in all sensitive growth stages on soil with AWC = 70 or 110 mm, respectively.
IV = Irrigation at 35 or 55 mm deficit in the first and at 52.5 or 82.5 mm deficit in the second sensitive growth stage, on soil with AWC = 70 or 110 mm, respectively.
2) In excess of that without irrigation.

Table 14. The effect of avaiable water capacity (AWC) on the frequency (% of years) of yield reductions in the absence of irrigation, relative to irrigation whenever deficits excee

	AWC	Yield reduction without irrigation <6%	6-15%	16-25%	>25%
CEREALS	70 mm	32	16	26	36
	110 mm	48	28	12	12
POTATO	70 mm	32	16	20	36
	110 mm	56	16	0	28
CARROT	70 mm	24	24	16	36
	110 mm	52	20	8	20
ONION	70 mm	36	16	12	36
	110 mm	52	24	12	12

irrigation rose to about one-third.

Differences between soils in the amount of irrigation water required, and in the amount lost to drainage, were relatively small compared with the divergence these variables showed between different irrigation strategies at the same AWC-value.

SUMMARY

1. Field trials were performed on a morainic loam soil to determine optimum irrigation strategy in cereals, potatoes, carrots and onions.

2. Studies of root distribution and water uptake suggested that the rooting zone was restricted to about 60 cm, and that water availability (AWC) corresponded approximately to the pF ranges 2-4.2 at 0-40 cm and 2-3 at 40-60 cm depths.

3. Good agreement was found between apparent evapotranspiration and values calculated using a soil moisture budget model.

4. Regression equations were derived of relative yield on relative evapotranspiration at different growth stages.

5. These equations were used to predict the effects of various irrigation strategies on yield levels, water use and drainage losses, over a twenty-five-year period.

6. Predicted mean maximum responses to irrigation on soil with 110 mm AWC were 21% for potato, 19% for carrot and 12% for both cereals and onion. Corresponding figures for soil with 70 mm AWC were 44%, 32% and 26%.

7. Non-irrigated yields were within 6% of the maximum obtainable level in about half the total number of years for soil with 100-110 mm AWC, and in about one-third of all years for soil with 70 mm AWC.

8. Non-irrigated yield levels were, in all crops, lower than 75% of the maximum obtainable in 36% of all years on soil with 70 mm AWC. Corresponding figures on soil with 110 mm AWC were 28% in the case of potato, 20% for carrot and only 12% for cereals and onion.

9. An irrigation strategy which allows soil moisture deficits to reach 50% of the AWC before irrigation appeared on average to give yield levels within 3-5% of the maximum obtainable.

10. Irrigation at 50% depletion of soil AWC in early growth stages, and at 75% depletion later on, reduces the amount of water necessary, as well as potential losses to drainage, by about one half, relative to irrigation at 25% depletion.

ACKNOWLEDGEMENTS

This work was financed by a grant from the Agricultural Research Council of Norway. The author is grateful to Svein Selnes for technical assistance and fieldwork.

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APPENDIX I

Calibration of the Troxler 3320 depth-moisture gauge

In order to use the neutron-probe in both top and subsoil, calibrations were performed in 200 liter drums (diam. 80 cm) using uniformly packed soil. Several soils were used to give a wide range of moisture contents.

The decline in relative count rates (RCR) close to the soil surface is shown in fig. 1a. Depths refer to the position of the neutron source, which is situated half-way up the probe. Soil moisture (SM) determinations were made gravimetrically from cylinder samples taken around the probe. The following calibrations were found:

Depth of source	Equation	R^2
5 cm	SM = 74.3 RCR + 2.6	0.97
10 cm	SM = 55.2 RCR + 1.2	0.99
15 cm	SM = 51.9 RCR - 0.6	0.99
20 cm	SM = 51.0 RCR - 1.5	0.99
>20 cm	SM = 49.9 RCR - 1.7	0.98

The spread of data for source depths of 5, 10 and >20 cm is illustrated in fig. 1b. The latter showed good agreement with the third-order equation determined artificially in the factory. The variations in bulk density and organic matter content between soils did not affect the linearity of the calibrations.

The influence of stones was investigated by distributing stones within 20 cm of the access tube, such that 80% of the volume within that radius was occupied by moist soil. The probe readings are expressed as percentages of the moisture content in the stone free soil (fig. 2a).

A greater decline in apparent moisture content was found near the surface than in deeper layers. The decline was almost proportional to the volume of stones for readings made at 5 cm, but was less than half that figure at depths greater than 20 cm. The greater «sphere of influence» caused by the presence of stones results in an increased loss of neutrons to the atmosphere when readings are made near the surface. Readings made deeper in the soil are simply averaged over a larger volume.

The sphere of influence of the neutron-probe was estimated by placing a layer of dry gravel in a profile of moist loam. This affected probe readings made within 10 cm of the layer (fig. 2b).

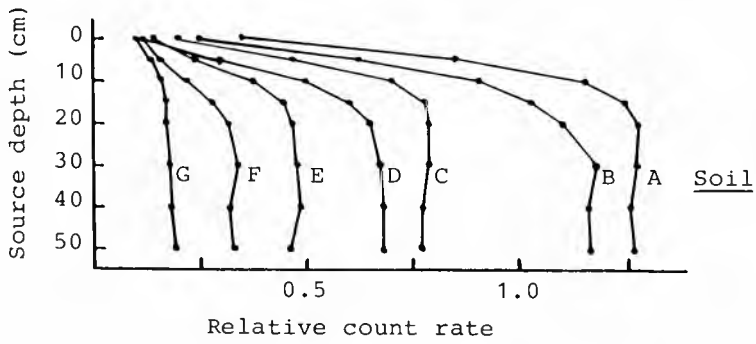
Readings made at the centre of the layer were about half the value of those made in the moist loam. Readings at 10 cm above or below the gravel were unaffected by its presence. The mean sphere of influence may therefore be taken as 20 cm. This was confirmed by measurements made in alternate layers of moist and dry soil (fig. 3).

CONCLUSION

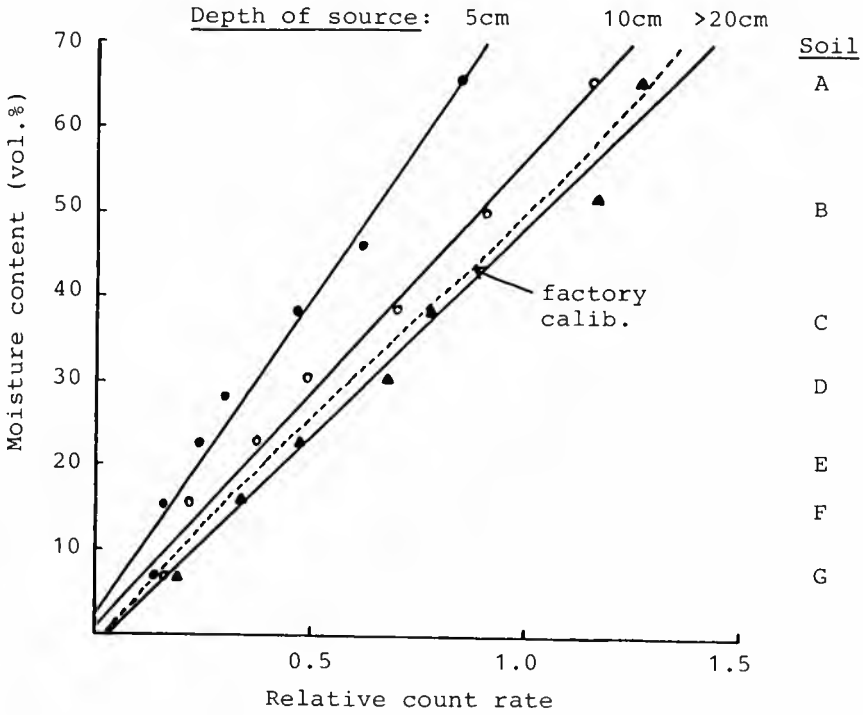
Satisfactory calibrations were obtained in both top and subsoil. The presence of stones reduces the absolute level of soil moisture measured, especially in the topsoil, but repeated measurements in same profiles nevertheless give realistic information on changes in soil moisture.

The mean «sphere of influence» has a diameter of about 20 cm. Readings at 5 cm depth give little additional information to those made at 10 cm. In order to obtain maximum information on water extraction patterns in the present study, readings were made at 10 cm intervals from 10 to 70 cm, and thereafter at 20 cm intervals down to 110 cm.

a)



b)



Soil	Texture	Ignition-loss	Bulk density	Moisture (vol.%)
A	Loam	16.4	0.92	66
B	Loam	16.4	1.07	52
C	Clay loam	3.0	1.79	38
D	Loam	16.4	0.93	30
E	Clay loam	3.0	1.54	23
F	Clay loam	3.0	1.52	16
G	Silt	3.0	1.17	7

Figure 1. Relative count rates at different source depths (above) and calibrations for source depths of 5 cm, 10 cm and >20 cm (below)

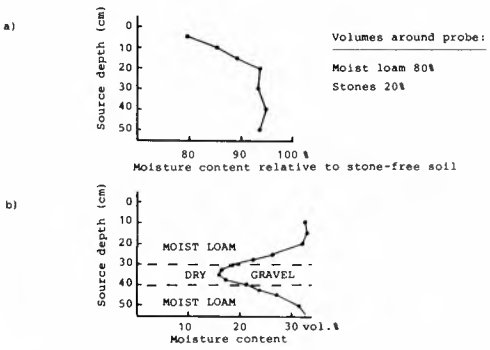


Figure 2. The influence of stones placed within a 20 cm radius of probe on moisture readings (above), and of a 10 cm layer of dry gravel (below)

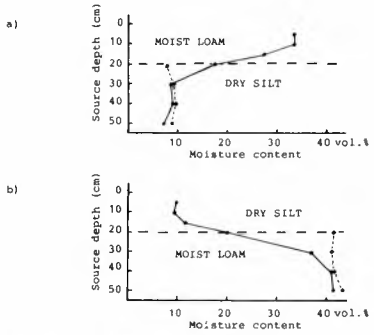


Figure 3. The influence of alternate layers of dry/moist soil on probe moisture readings in adjacent layers. Open circles denote original values

APPENDIX II

Estimation of potential evapotranspiration at Kise Research Station

The Penman equation is the most universally accepted means of calculating potential evapotranspiration (Ep). Numerous comparisons with the results of this equation have been performed throughout Scandinavia, both with data derived from other equations, data from evaporation pans and data from grass lysimeters. Many of these studies have shown that such estimates of Ep give slightly lower (approx. 10%) overall values than the Penman method, and exhibit a marked seasonal divergence, with lower values in spring and higher values in autumn, possibly influenced by the direction of heat flow in soil or water.

Data is presented here of Ep at Kise Research Station over four years (1979-1982), calcuated by means of four different equations and measured directly using three different evaporimeters. This information is given for comparative purposes, and no indication of the relative merits of the various methods is intended.

Average weather data for the four years is given in Table 1. Mean monthly evaporation (mm day^{-1}) is given in Table 2, together with relative values (% of Penman) in individual years. The Penman estimates exhibit the same seasonal trend as mentioned above, relative to the values obtained by direct measurement. The equations of Hansen (1980) and Aslyng and Hansen (1982) yielded similar values to that of Penman eguation (1963), whilst the equation of Johansson (1969) gave values closer to those measured with the Thorsrud evaporimeter.

The Thornthwaite grass lysimeter and Andersson evaporimeter showed considerably greater between-year variation than other methods of estimation. Both gave higher Ep than the Penman method in the warm, dry con di tions of 1982, possibly due to an 'oasis' effect.

Regressions of daily Ep values on solar radiation and an advection term, as per formed by Johansson (1969), yielded the following equations from the present data:

$$Ep\,(Andersson) = 4.15 \times 10^{-3} Rs + 0.26u(es-e) - 0.23 \quad (R^2 = 0.78)$$

$$Ep\,(Thorsrud) = 2.77 \times 10^{-3} Rs + 0.14u(es-e) + 0.44 \quad (R^2 = 0.53)$$

where: Rs = solar radiation (cal.cm^{-2}day^{-1})
u = mean windspeed at 1.5 m (m sec^{-1})
(es-e) = vapour saturation deficit (mm Hg)

Table 1. Weather data over four years at Kise Research Station (May to September)

	1979	1980	1981	1982	Normal
Air temp. (deg.C)	11.9	12.9	12.2	13.0	12.5
Solar rad.(cal.cm^{-2}day^{-1})	357	361	352	397	384
Relative humidity (%)[1]	71	70	71	64	74
Windspeed (km day^{-1})	156	102	101	120	124
Relative sunshine (%)[2]	39	39	36	45	39
Precipitation (mm)	281	344	258	222	290

[1] Mean of 06,12 and 18 hrs. GMT
[2] Percent of maximum possible for latitude

Table 2. Potential evapotranspiration over four years at Kise Research Station

Method No.		Monthly means (mm day^{-1}) May	June	July	Aug.	Sept.	Mean
Equations							
1 Penman 1963		2.57	3.28	3.17	2.29	1.18	2.50
2 Hansen 1980		2.60	3.30	3.13	2.26	1.19	2.50
3 Aslyng et al.1982		2.73	3.26	3.26	2.43	1.35	2.61
4 Johansson 1969		2.30	2.61	2.39	1.99	1.37	2.13
Evaporimeters							
5 Thornthwaite[1]		2.14	3.15	2.93	2.75	1.55	2.52
6 Andersson 1969		2.42	3.02	2.85	2.54	1.71	2.52
7 Thorsrud[1]		2.02	2.64	2.43	2.34	1.39	2.17
		Annual variation (relative to Penman)					
Year	Method No.1	No.2	No.3	No.4	No.5	No.6	No.7
1979	100(=2.45mm)	103	101	90	86	100	87
1980	100(=2.43mm)	99	106	83	92	88	84
1981	100(=2.34mm)	99	105	82	99	100	83
1982	100(=2.79mm)	100	105	86	122	111	91
Mean	100(=2.50mm)	100	104	85	100	100	87

[1] Described in Hetager and Lystad 1974.

Compared with Johansson's equation, which itself was based on measurements made with an Andersson evaporimeter, these results indicate a higher sensitivity of the Andersson evaporimeter at Kise, particularly to variations in advection, and a lower sensitivity of the Thorsrud evaporimeter to variation in solar radiation. The latter effect, together with the high constant term in the Thorsrud equation, reflect the high heat capacity of this evaporimeter, which causes a buffering of short-term fluctuations.

CONCLUSION

The Thorsrud evaporimeter, upon which the results of the present study are based, normally gives about 10-15% lower Ep values than those calculated according to Penman (1948) and some other commonly used equations. On the other hand, it shows close agreement with values obtained using the equation of Johansson (1969), which was derived under similar climatic conditions in Sweden. Comparisons with grass lysimeter values have given no conclusive

support to any particular method of estimation.

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EFFECT OF WEIGHT LOSS ON SUSCEPTIBILITY TO *BOTRYTIS CINEREA* IN LONG-TERM STORED CARROTS

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The susceptiblity to *Botrytis cinerea* infection in long-term stored carrots is highly influenced by weight loss. The critical weight loss which leads to infection in slowly dried carrots is around 5%.

Key words: *Botrytis cinerea*, Carrots, Cold storage, Disease susceptibility, Weight loss.

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When carrots are kept in cold stores over a long period a substantial weight loss can take place unless they are wrapped in plastic. When carrots lose turgor, there is an increase in susceptibility to *Botrytis cinerea* (Årsvoll 1969, Heale et al. 1977). Goddliffe & Heale (1977) concluded that a fresh weight loss of over 5% leads to an increase in *B.cinerea* infection. Their experiments were carried out on rapidly dried carrots (1-2% per day) which were inoculated with the pathogen. It is not known, however, if the same "critical" weight loss limit applies to carrots which lose water more slowly. The present work was initiated to investigate to what extent carrots which lose water slowly are susceptible to fungal infection.

MATRIAL AND METHODS

Carrots *Daucus carota* L. var Nantes Duke, were sown in May at Aas. No fungicides were used, but chemical control of pests (diazinon) and weeds (linuron) was carried out. The carrots were harvested by hand in October, placed in polyethylene nets (12 kg/net) and stored in an icebank cooler in wooden bins with slatted bottoms and walls covered with polyethylene at 0.7 to 1.0 °C, or wrapped in polyethylene and placed in a cold store at 0 °C (Tronsmo & Hoftun 1984). To obtain material with different weight losses for the inoculation experiments, some nets were either stored on the floor in the icebank or left unwrapped in the cold store for one to four weeks.

The unwashed carrots were taken out of the cold stores and the average weight loss determined. Carrots without disease symptoms were either wrapped individually in plastic bags (control) or, before wrapping, dipped in a condidial suspension of *Botrytis cinerea* Pers. (10^5 cfu/ml). The *B. cinerea* conidia (from culture *B. cinerea* 208, an isolate from carrot) was harvested from 14-day-old cultures grown on a 2% malt extract agar. The control and inoculated carrots were stored at 5 °C, and the disease assess-

ments were carried out after 4, 8 and 12 weeks.

The other roots were gently washed in cold tap water, surface sterilized in 70% ethanol for one minute, and then rinsed in sterile water. The carrots were diced either transversally to give 5 mm thick discs, or longitudinally into 5 cm sections with a maximum thickness of 7 mm. The pieces were then placed (the longitudinal ones with the undamaged surface upward) in a humid chamber made in 9 cm petri dishes with a cellulose filter paper dampened with 0.8 ml of sterile water. The weight of the carrot pieces was controlled during the experiment, and it never exceeded a 3% change in weight.

The carrot pieces (six parallels in each group) were inoculated with mycelial discs (5 mm dia.) taken from the periphery of actively growing *B. cinerea* 208 colonies on malt agar plates. The discs were placed with the fungal mycelium in contact with the cut carrot surface or the intact undamaged surface of the longitudinally cut sections. The plates were incubated in the dark at 4 °C for up to 14 days.

RESULTS AND DISCUSSION

The inoculation of carrot pieces with agar discs of *B. cinerea* showed that the fungus was able to grow on the carrot surface and cause rot. The growth rate was much faster on the undamaged surface than on the cut surface and may have been due to phytoalexin production on the cut surface (Goddlife & Heale 1978). However, the brown rot development was faster on the cut surface. Carrots with a water loss from 1 to 16 % were tested, but there was no significant effect of water loss on growth and rot development. This indicates that inoculation of carrot discs is not a suitable method for investigating change in susceptiblity to *B.cinerea* attributable to water loss.

Table 1 shows the effect of artificial infection by *B.cinerea* conidia on undaged carrots after 6 to 34 weeks stor-

Table 1. The effect of weight loss on *Botrytis cinerea* attacks on cold-stored carrots. Percent age of carrots with *B. cinerea* rot with standard error of mean (8 carrots and 4 parallels in each group) 12 weeks after infection.

Weeks in store before infection	% Weight loss at infection	% rot	
		Control	*B. cinerea* infected
6	0	0	0
6	7.3	0	6 ∓ 4
6	15.7	15 ∓ 9	33 ∓12
14	2.5	0	3 ∓ 2
14	7.0	0	0
14	12.8	0	17 ∓ 7
14	30.5	0	20 ∓12
28	4.4	0	0
28	12.6	13 ∓ 6	6 ∓ 3
28	25.6	51 ∓14	43 ∓ 9
34	5.0	0	0
34	9.3	0	0
34	14.5	0	42 ∓ 6
34	25.0	0	6 ∓ 5

age. The infection by *B. cinerea* shows significant correlation with the weight loss. However, the disease assessment on carrots stored for the longest period may have been underestimated because of severe infection by *Mycocentrospora, acerina* which may have masked simultaneous infection by *B. cinerea.*

Figure 1 shows the correlation between the weight loss and the natural development of *B. cinerea* rot on carrots stored for 9 months in an icebank cooler or cold store during a 6-year experimental period. There is a clear connection between weight loss and infection by *B. cinerea*, even though the extent of the disease caused by *B. cinerea* varies from year to year. This can partly be explained by infection by other storage rotting organisms (*Mycocentrospora acerina, Sclerotium sclerotiorum)* which may mask simultaneous infection by *B. cinerea*. The critical weight loss on slowly dried, naturally infected, longterm stored roots, was around 5%, which is in good agreement with published experiments (Goodliffe & Heale 1977). This indicates that there is a fundamental increase in susceptiblity to *B. cinerea* when weight loss is over 5%, and that slow drying, which would more readily allow internal equilibration of turgor levels, does not affect the increase in susceptiblity to fungal infection.

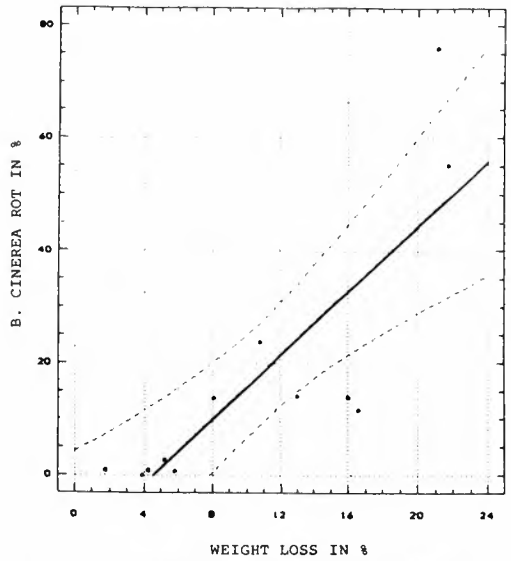

Figure 1. Correlation between *Botrytis cinerea* rot and weight loss. The data are from 6 years experiment where three times 12 kg carrots were stored in an icebank cooler or cold store for 9 months. The dotted lines indicate the 95% confidence interval.

ACKNOWLEDGEMENT

Technical assistance from Sylvia Bredholt, storage facilities made available by Department of Vegetable Crops, and financial support from the Agricultural Research Council of Norway are all gratefully acknowledged.

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EFFECT OF FUNGICIDES AND INSECTICIDES ON GROWTH OF *BOTRYTIS CINEREA*, *TRICHODERMA VIRIDE* AND *T. HARZIANUM*

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Tronsmo A., Effect of fungicides and insecticides on growth of *Botrytis cinerea*, *Trichoderma viride* and *T. harzianum*. Norwegian Journal of Agricultural Sciences 3: 151-156. ISSN 0801-5341.

The effects on growth and on tolerance to commercial agrochemicals used against diseases and pests on fruit and berries were tested on *Botrytis cinerea*, *Trichoderma viride* and *T. harzianum*. There was some difference in sensitivity between *B.cinerea* and the two *Trichoderma* species. In most cases, however, the difference was too small to be of use in an integrated control against *B.cinerea* with *Trichoderma* spp. as antagonist. Insecticides also highly inhibit fungal growth, and on the average the insecticides were at the recommended concentration, as or more inhibitory to the test fungi than the fungicides.
Tolerance against agrochemicals was often found in *Trichoderma* spp. and these isolates seldom showed any reduced growth ability or increased sensitivity to high osmotic pressure compared with the parent strain. Tolerant and competitive antagonistic isolates of *Trichoderma* spp. are therefore suitable for integrated control programs.

Key words: Antagonists, Biological control, Fungicides, Insecticides, Integrated control, Resistance, Tolerance.

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Despite careful selection, many fungicides and insecticides with high biological activity tend to have some effects on nontarget organisms. Undesirable side-effects are common (RodriguezKabana & Curl 1980, Griffiths 1981) and these include the induction of «new» diseases or the increased severity of diseases already present.

The reason for the increase in disease severity through the use of some crop protection chemicals, the so-called iatrogenic diseases (Horsfall 1972), is not always known. However, some cases of increased disease severity have been documented as being caused by the disturbance of natural antagonists such as *Trichoderma viride*, an antagonist to root pathogens (Baker & Cook 1974) and *Penicillium brevicompactum*, an antagonist to *Botrytis cinerea* on cyclamen (Bollen 1971).

Another danger with using fungicides in disease control is the possibility of selecting fungicide-resistant pathogens in the field. This problem was first described in *Botrytis cinerea* against Benomyl (Bollen & Scholten 1971), but it has now become a major problem (Cook & Baker 1983).

This paper presents an investigation of the effects of insecticides and fungicides on fungal growth. The aim was to select antagonists that could be used in an integrated control program against fungal diseases. As a model system, *Botrytis cinerea*, a pathogen that attacks nearly all weakened plant parts (Coley-Smith et al 1980), and isolates of *Trichoderma* spp., a well-known antagonist to plant pathogens and other fungi (Dennis & Webster 1971, Baker & Cook 1974, Tronsmo & Dennis 1978, Cook & Baker 1983, Tronsmo 1986), were chosen.

MATERIALS AND METODS

Isolates of *Trichoderma viride* Rifai and *T. harzianum* Rifai were from the culture collection at the Food Research Institute, Norwich, England, and our institute. The fungi were originally isolated from soil (*T. viride* 1 and 109, *T. harzianum* 187), wood (*T. harzianum* 103 and 107), from the saprophytic leaf flora of strawberry leaves (*T. harzianum* 169 and 207), and the rhizosphere of carrots (*T. harzianum* 196). *Botrytis cinerea* Pers ex Fr. was isolated from apple fruit with Dry eye rot.

The fungi- and insecticides used in this trial were commercial agrochemicals used against diseases in fruit and berries in Norway.

The fungi isolates were tested for growth on malt agar plates (5.0 g malt extract, 5.0 g glucose, 0.5 g NH_4NO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15.0 g agar in 1000 ml water). When pesticides was included in the media, they were dissolved in steril distilled water and then added to the warm autoclaved agar (50°C). The concentrations of the agrochemicals tested were from 0.1 mg l^{-1} to 1000 mg l^{-1} or, when higher, to the concentration recommended for field use in Norway. The newly-made plates were incubated with mycelial discs (5 mm diam.) taken from the periphery of actively growing colonies on malt agar plates. The discs were placed with the fungal mycelium in contact with the agar surface. The plates were incubated in the dark for 48 hours at 21°C. Two diameters at right angles were measured on each of two replicate plates, and the concentration which inhibited mycelial growth by 50% (ED 50) was calculated.

Tests for osmotic sensitivity were performed on malt agar plates with 0, 5, 10, 20 and 50 g l^{-1} glucose and on ordinary malt agar with 15, 33.5 and 70 g l^{-1} KCl. Two diameters at right angles were measured on each of two replicate plates, and the growth as precentage of growth without glucose was calculated.

RESULTS AND DISCUSSION

Table 1 shows the effect of 33 agrochemicals on growth of *Botrytis cinerea* and eight *Trichoderma* isolates. The concentration of the active ingredient that causes a 50% reduction in growth (ED 50) and the % inhibition at the recommended concentration (of active ingredient) (IRC) are given. There is a marked difference in sensitivity to agrochemicals between *B. cinerea* and the tested *Trichoderma* isolates, whereas the differences in sensitivity between *Trichoderma* species are smaller. As expected, *B. cinerea* is not only more sensitive than *Trichoderma* to fungicides recommended for use against diseases caused by *B. cinerea* (Benomyl, Iprodione, Dichlofluanid, Tolylfluanid and Vinclozolin), it is also more sensitive to the fungicides Mancozeb, Propineb, Triadimefon and the insecticides Bromophos and Malathion. On the other hand, *Trichoderma* spp. is more sensitive to the fungicides Bitertanol, Captafol, Captan, Copper oxychloride, Dodine and Triforine, and the insecticides Azinphos-methyl, Dicofol and Dimethoate. These results were confirmed in more detailed study on 14 agrochemicals and four fungi (Table 2).

Table 1. Effect of fungicides and insecticides on growth of *Botrytis cinerea* 2 (B.c.), *Trichoderma viride* (Tv 1, Tv 109) and *Trichoderma harzianum* (Th 107, Th 103, Th 207, Th 169, Th 187, Th 196). Concentrations of active ingredients needed for 50% inhibition of mycelial growth (ED 50) and inhibition of growth at recommended concentration (IRC) are given

Common name of active ingredient	Trade mark	Recommended concentration in ppm	Bc		Tv1		Tv109		Th107		Th103		Th207		Th169		Th187		Th196	
			ED50	IRC	ED50	IRC	ED50	IRC	ED50	IRC	ED50	IRC	ED50	IRC	ED50	IRC	ED50	IRC	ED50	IRC
FUNGICIDES																				
Benomyl	Benlate	250	<1	100	1	100	1	100	3	100	3	100	<1	100	1	100	<1	100	<1	100
Binapacryl	Acricid 50	480	100	80	100	85	100	85	300	75	200	75	100	65	100	85	50	85	50	85
Bitertanol	Baycor 25 wp	250	15	50	6	70	7	70	4	65	2	70	4	70	3	75	3	75	3	75
Captafol	Difolatan 80	640	10	70	1	95	<1	95	2	90	2	90	10	80	<1	100	1	95	1	90
Captan	Orthocid 83	1275	85	80	5	95	10	95	25	70	25	70	5	90	10	95	10	95	5	95
Captan	Orthocid 10	Dustem	10	-	10	-	10	-	100	-	100	-	10	-	3	-	5	-	3	-
Chinomethioat	Morestan	125	250	55	125	50	250	55	>250	35	>250	35	>250	35	>250	45	>250	40	>250	45
Copper oxychloride	Kopperkalk	2100	800	50	40	100	30	100	65	95	80	90	300	85	40	100	250	100	35	90
Dichlofluanid	Euparen	2000	<1	100	15	90	15	90	50	85	50	95	10	90	10	90	10	95	10	95
Dinocap	Karathane	225	3	100	2	90	2	90	4	85	4	90	3	85	2	90	2	85	3	90
Dodine	Melprex 65	390	400	65	40	100	40	100	65	90	65	90	50	100	65	100	50	100	50	100
Dodine	Syllit	378	60	100	20	100	25	100	25	95	20	95	20	100	20	100	15	100	20	100
Iprodione	Rovral	750	<1	100	1	100	1	100	2	85	2	80	3	75	1	100	1	100	1	100
Mancozeb	DithaneM-45	1600	80	100	800	50	800	55	800	45	800	45	800	60	800	50	1600	45	1600	45
Propineb	Antracol	1050	280	55	1000	50	1050	50	>1050	35	>1050	35	>1050	35	>1050	35	>1050	35	>1050	45
Sulphur	Bayer Svovel	5600	>5600	20	>5600	25	>5600	25	>5600	25	>5600	20	>5600	15	>5600	30	>5600	35	>5600	30
Thiram	Pomarsol	1600	1	100	10	80	10	80	3	80	2	70	1	100	4	100	2	90	<1	90
Tolylfluanid	Euparen M	2000	0,5	100	13	85	15	85	50	90	40	80	50	85	13	95	20	90	5	90
Triadimefon	Bayleton	125	15	95	100	65	100	70	125	65	100	65	50	65	50	70	75	65	75	60
Triforine	Saprol	285	15	100	2	100	2	100	6	100	6	100	10	100	2	100	2	100	2	100
Vinclozolin	Ronilan	750	<1	100	1	95	1	100	3	85	2	85	3	80	1	95	1	95	<1	100
INSECTICIDES																				
Azinphos-methyl	Gusation	375	375	40	100	60	250	60	250	60	250	60	175	70	150	65	175	60	250	65
Bromophos	Nexion 40	400	10	100	20	100	20	100	20	90	20	90	20	100	20	95	20	95	20	100
Demethon-S-methyl	Meta-systox	250	25	70	25	65	25	60	75	60	250	55	250	50	25	65	100	60	40	70
Diazinon	Basudin 25	230	20	100	10	100	10	100	20	95	20	100	20	100	15	100	20	95	25	95
Dicofol	Kelthane	370	30	75	5	100	5	100	10	90	10	90	10	95	10	90	10	90	5	95
Dimethoate	Rogor L 20	300	200	75	80	85	80	80	80	85	80	85	80	80	80	85	60	85	80	85
Endosulfan	Thiodan	535	10	85	35	95	10	95	70	85	70	90	35	90	70	85	70	90	35	90
Fenitrothion	Folithion	750	5	100	5	100	10	100	20	90	20	90	20	100	15	90	15	90	15	100
Fenitrothion	Sumithion	795	15	100	10	95	10	95	25	90	20	90	20	90	5	90	25	85	20	90
Fenthion	Lebaycid	800	30	85	35	75	20	75	45	80	45	80	30	75	40	80	25	80	30	85
Malathion	Malathion	1000	20	85	50	100	50	100	100	95	100	95	100	95	50	100	50	90	50	90
Parathion	Bladan	140	20	60	35	55	35	60	35	55	35	55	40	55	35	55	40	55	35	60

Table 2. Effect of agrochemicals on growth of *Trichoderma viride* 1 (Tv 1), T. harzianum 107 (Th 107), *T. harzianum* 169 (Th 169), and *Botrytis cinerea* (B.c). Concentrations in ppm of active ingredients giving 25%, 50% and 75% (LD25 LD50 LD75) inhibition of of growth are given.

Common name of active ingredient	Trade mark	LD25				LD50				LD75			
		Tvl	Th107	Th169	B.c	Tvl	Th107	Th169	B.c	Tvl	Th107	Th169	B.c
FUNGICIDES													
Benomyl	Benlate	0.4	1.5	0.3	0.2	1	3	1	0.5	1.5	4	1.5	0.8
Binapacryl	Acricid 50	13	13	8	13	90	300	100	110	500	>500	500	500
Bitertanol	Baycor 25	0.4	1	0.5	0.8	6	4	3	15	>250	>250	>250	>250
Dichlofluanid	Euparen	5	5	2	0.2	30	100	18	1	400	1000	100	4
Dinocap	Karathane	0.5	1	0.3	1	2	4	2	3	20	45	20	70
Dodine	Syllit	6	12	6	25	10	20	20	200	25	60	30	500
Dodine	Melprex 65	16	20	20	20	40	65	65	400	45	130	130	>650
Iprodione	Rovral	0.5	1.5	0.5	0.2	1	2.5	1	0.5	1.5	13	2	1
Tolylfluanid	Euparen M	1	3.5	2.5	0.3	13	50	13	0.5	150	250	100	1.5
Triadimefon	Bayleton 25	25	40	8	5	100	125	50	15	>250	>250	250	50
Triforine	Saprol	1	4	1	4	2	6	2	15	5	11	6	60
Vinclozolin	Ronilan	0.5	1.5	0.5	0.1	1	2.5	1	0.2	1.5	5	2	0.3
INSECTICIDES													
Bromophos	Nexion 40	6	6	4	1	20	20	20	12	40	80	60	40
Diazinon	Basudin 25	6	7	5	6	10	20	15	20	45	70	60	70

The difference in sensitivity between the *Trichoderma* spp. isolates are less than between *Trichoderma* and *B. cinerea*, although some differences can be found. The wood isolates *T. harzianum* 103 and 107 are more resistant to Benomyl, Binapacryl, Captan and Dichlofluanid than the other *Trichoderma* isolates (Table 1), and *T. harzianum* 107 is equally or more resistant than *T. viride* 1 and *T. harzianum* 169 to all the 14 tested chemicals in Table 2.

Tolerance of agrochemicals was often found in *Trichoderma* spp. On many of the pesticide-amended plates, especially those with high concentrations of chemicals, unexpected fast growth compared with the growth rate at lower concentrations was seen either in the whole colony or more often in a sector. This was most frequently seen on Vinclozolin, Ipodione, Bitertanol and Copper oxychloride plates, but also on Benomyl and Dichlofluanid amended plates. A typical example of resistance to dicarboximide fungicide is shown in Fig 1, where 50 and 500 ppm Vinclozolin are apparently less inhibitory than 12 ppm. Such isolates were further tested for viability and tolerance of pesticides. Some of the isolates died, but most of them retained the increased tolerance for more than 10 transfers to new plates without pesticides, which indicates a genetically stable tolerance.The growth

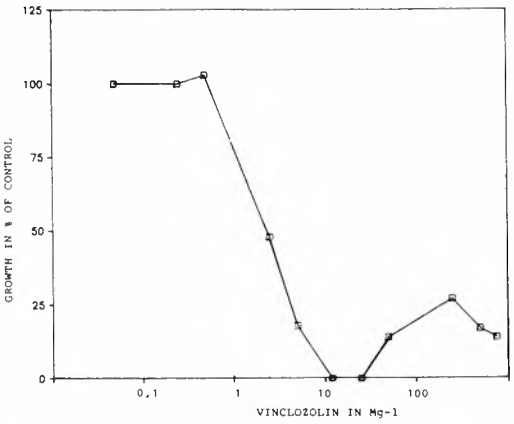

Figure 1. Effect of Vinclozolin on growth of *Trichoderma harzianum* 107

rate on malt agar plates was also the same for many of the tolerant isolates at different temperatures.

Beever (1983) has shown that many dicarboximide-tolerant isolates of *B. cinerea*, *Penicillium expansum* and *Aspergillus nidulans* were more inhibited by the addition of 0,68 M sodium chloride to the basal malt/pepton media than the parent strain. Of four tolerant strains of *T.harzianum* none was more inhibited by up to 50 g/l of glucose or 35.5 g/l potasium chloride, than the parent strain. This indicates that the tolerant strain should have no less competitive ability than the parent strain on nutrient-rich plant surfaces.

In the tolerant isolates, cross-resistance to non-related chemicals is often found. The growth of four *T. harzianum* isolates on Dichlofluanid, Ipodione and Vinclozolin amended plates is shown in Fig. 2. All the isolates selected from the pesticide plates showed much higher tolerance of the pesticides than the parent strain, and there was a slightly lower tolerance against dicarboximide fungicides in the isolate from Dichlofluanid amended plates.

The changes in tolerance are so frequent in the cases of Vinclozolin and Iprodion in *Trichoderma* spp. that they are often difficult to demonstrate, as both the isolate from the fungicide plate and the control isolate showed this unexpected high growth rate.

In *B. cinerea*, resistance to Benomyl, Iprodione and Vinclozolin is well documented (Bollen & Scholten 1971) (Leroux et al. 1977, Dennis & Davis 1979) and is also frequently found in this study.

The easily obtained and stable tolerance found in this study has indicated that antagonistic *Trichoderma* isolates may be suitable for an integrated control program against fungal plant diseases. However, the difference in tolerance between wild types of *Trichoderma* and the pathogen *B. cinerea* is not sufficient to suggest wild types of *Trichoderma* in an integrated spray program. A more promising approach would be to select pesticide tolerant and highly competitive antagonistic *Trichoderma* isolates and use them in an integrated control program.

In integrated spray programs with selected fungal antagonists, the effect on the biocontrol agent of the fungicides used in the field would have to be investigated. In addition, a careful investigation of the effects of other agrochemicals would have to be made, as Table 1 shows marked inhibition of both *B. cinerea* and *Trichoderma* spp. by insecticides. On average, the insecticides at the recommended concentration are even more inhibitory to the tested fungi than the fungicides (Table 3). To obtain a successful integrated spray programs, close cooperation between different dici-

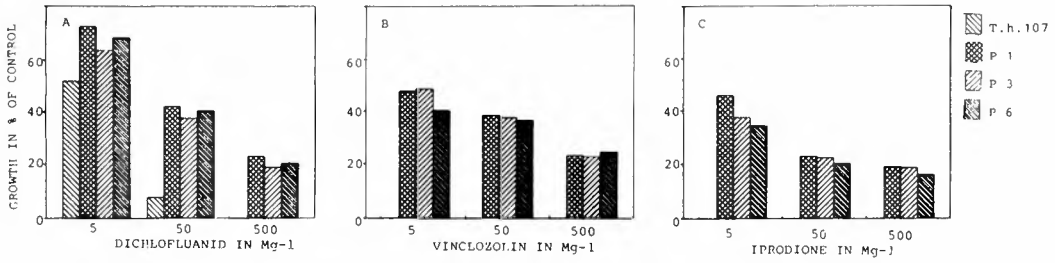

Figure 2. Effects of Dichlofluanid, Vinclozolin and Iprodione on growth of *Trichoderma harzianum* (Th 107) and tolerant isolates isolated from plates amended with Iprodione (P1), Vinclozolin (P3) and Dichlofluanid (P6)

Table 3. Average inhibition at the recommended concentration (See Table 1) of 21 fungicides and 12 insecticides

	% inhibition of growth								
	Bc	Tv1	Tv109	Th107	Th103	Th207	Th169	Th187	Th196
Fungicides	80	81	82	73	73	75	82	81	81
Insecticides	81	86	85	81	81	83	83	81	87

plins in plant protection is therefore needed.

ACKNOWLEDGEMENT

The author thanks J. Postma and S. Dahl for their skilful technical assistance.

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TRICHODERMA HARZIANUM USED FOR BIOLOGICAL CONTROL OF STORAGE ROT ON CARROTS

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Tronsmo, A. 1989. *Trichoderma harzianum* used for biological control of storage rot on carrots. Norwegian Journal of Agricultural Sciences 3:157-161. ISSN 0801-5341.

Storage rot on carrots caused by *Botrytis cinerea*, *Mycocentrospora acerina*, *Rhizoctonia* carotae and *Sclerotium sclerotiorum* was reduced during long term storage at 0°C by dipping the roots after harvest in a conidial spore suspension of the antagonistic fungus *Trichoderma harzianum*.

Key words: Antagonist, Biological control, *Botrytis cinerea*, Carrots, Cold storage, *Mycocentrospora acerina*, *Rhizoctonia* carotae, *Sclerotium sclerotiorum*, Storage, *Trichoderma harzianum*.

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Biological control of fungal pathogens has attained increased attention over the last years. Most of the work has been concentrated on control of soil-borne pathogens in the field (Cook & Baker 1983), whereas less attention has been given to control of the crop after harvest. Wilson and Pusey (1985), however, point out that there should be good prospects for biological control of post-harvest rot if antagonists were found that would work under conditions suitable for storage of the crop.

Since most fruit and vegetables are preferably stored in cold room at about 0°C (Dennis 1984), this restricts the number of antagonists that can be used. However, antagonistic *Trichoderma* spp., which grows at low temperatures, can be selected (Tronsmo & Dennis 1978). One of these isolates, effective against dry eye rot on apple (Tronsmo 1986 a,b), was used in these trials in an attempt to control post-harvest rot on cold-stored carrots.

The main spoilage organism on carrots stored in open bins is *Botrytis cinerea* (Årsvoll 1968). However, this pathogen can almost be eliminated if the carrots are stored in bins lined inside with polyethylene. This is now commercial practice in Norway. However, rot caused by *Mycocentrospora acerina* and *Rhizoctonia carotae* has become more serious on polyethylenewrapped carrots. This is probably caused by increased availability of water and higher temperatures during the first part of the storage period. Even when holes are made at the top and bottom of the polyethylene sheets to prevent anaerobic conditions in the bins, temperature measurements in the middle of the bins have shown that the cooling rate of the carrots is markedly decreased by the insulation caused by the polyethylene wrapping (Tronsmo & Hoftun 1984).

MATERIALS AND METHODS

Fungi

The antagonist used in this study was *Trichoderma harzianum* (Rifai) P 1, a mutant of *T. harzianum 107* (Tronsmo & Dennis 1978) isolated from a malt agar plate containing 500 mg l^{-1} Iprodione. This isolate is tolerant to dicarboximid fungicides (Tronsmo 1989).

Fields

Carrots (*Daucus carota* L.) var. Nantes Duke were sown in May in two fields. One was at Aas, Akershus County, Norway, on sandy soil naturally infested by *Mycocentrospora acerina* (Hartig) Deighton, after continuous growing of carrots in the field for ten years, and the other at Hedemark County, Norway, a field with organic soil, where the previous years carrot crop had been heavily infected by *Rhizoctonia carotae* Rader during storage. No fungicides were used, but chemical control of pests (diazinon) and weeds (linuron) was carried out. The carrots were harvested by hand at Aas, and by toplifter at Hedemark.

Storage conditions

Preliminary experiments have shown that during long-term cold storage of carrots, less rot was obtained on unwashed than on washed carrots (Tronsmo et al. 1982). This storage procedure was therefore used in these experiments. On the day of harvest the unwashed carrots with traces of soil were placed in polyethylene nets (12 kg/net) and stored randomly in 400 kg bins which had the bottom, sides, and top covered with polyethylene to prevent the carrots from drying out (Tronsmo & Hoftun 1984).

Room temperature during the storage period was 0-0.5°C, but it took 2 months fore the temperature in the middle of the produce to come down to 2°C, where it stabilized for the rest of the storage period.

Biological control treatments

Before storage the *Trichoderma*-treated carrots were soaked for 5 min in a conidial suspension (10^7 conidia/ml) of *T. harzianum* P 1. This treatment caused an average surface coverage of 10^3 cfu *T. harzianum* P 1 per cm^2. The conidial suspension was made in the following way: 200 g barley grains were boiled for 45 min in 200 ml water and autoclaved 50 min. at 121°C in 25x38 cm autoclavable plastic bags (Polyethylene Terephtalate,ICI). The bags were inoculated with 50 ml of a conidial suspension of *T. harzianum* P 1 (10^8 conidia/ml) and incubated at 22°C. During the 10-day growing period the bags had to be shaken every day to obtain an even distribution of the fungus over the grains and to prevent clogging.

Disease assessment

At each registration of storage rot, three bags of each treatment with 12 kg carrots were examined and divided into the following groups: No visible disease symptoms, rot caused by *B. cinerea*, rot caused by *M. acerina*, rot caused by *R. carotae* and rot caused by other fungi. The rot caused by *B. cinerea*, *M. acerina* and *R. carotae* was usually determined by visual examination of the rot tissue, but when there was some doubt or when the rot was caused by other fungi, the casual organism was determined after incubation on malt agar plates at 22°C of a piece of tissue from the leading edge of the rot. The overall effect of the *Trichoderma* treatment on the different fungi was tested by two-way analysis of variance (ANOVA), using date of examination as blocks, and the frequency in each bag as single observations ($n=3$ for each treatment within each block). All the fields were included in the ANOVA (each date treated as a separate block, without correction for differences between field trials). In some cases, however, ANOVA was sum separately for each field trial.

Fungal and bacterial flora on the carrot surface

At regular intervals, four replicates containing 8 carrots with no visible disease symptoms, were removed for determination of the microbial surface flora. The free soil was washed off by gently hand rubbing in cold water, and about 700 g of carrots were grinded 90 sec. in a Weissen homogenizer type 81. Ten grams were then transferred to sterile Stomacher bags, diluted with 90 ml peptone water (Bactopeptone, Difco 10 g/l, NaCl, 5 g/l), and homogenized for 60 sec in a Cotworth Stomacher 400. Three 10-fold dilutions of each blended sample were made in peptone water; 0.1 ml samples were spread on Petri dishes containing Plate Count Agar (PCA, Difco) or Potato Dextrose agar (PDA, Difco) acidified with citric acid to pH 4.3. Two parallels of the proper dilutions were incubated aerobically at 4°C and 20°C (PDA and PCA) and in anaerobic jars with Oxoid gass generating kit and catalyst (PCA) at the same temperatures. The PCA plates were recorded after 7 and 3 days at 4° and 20°C, respectively. The PDA plates were recorded after 14 and 21 days at 4°C and 5 and 10 days at 20°C. The presence of one colony on the lowest dilution plates corresponds to a count of 100 viable units/g of carrots.

RESULTS AND DISCUSSION

The percentage of carrots with disease sympoms is shown in Fig. 1. In the figure it is shown that the *Trichoderma* treatment markedly increased the amount of uninfected carrots ($p = 0.0002$, ANOVA through all trials). On average, after about 6 months in cold storage the amount of marketable crop had increased by 47 % and after 8 1/2 month by 75% by the *Trichoderma* treatment.

The percentage of rot caused by *B. cinerea*, *M. acerina*, *R. carotae* and *Sclerotium sclerotiorum* (Lib.) De Bary on the control and *Trichoderma*-treated carrots is shown in Fig. 2. The figure shows that there was a significant reduction in the attack by three of the pathogens after the *Trichoderma* treatment. (The ANOVA test for the three organisms were: *B. cinerea* $p = 0.02$, *R. carotae* $p = 0.09$, *S. sclerotiorum* $p = 0.12$).

These experiments were repeated in both fields in 1985/86, but no significant effects of the treatment could be seen. This could have been caused by a mutation in the formerly cold-tolerant selected mutant, as re-isolation from the carrots showed that the antagonist was unable to grow below 5°C. This problem of mutation in *Trichoderma* has to be

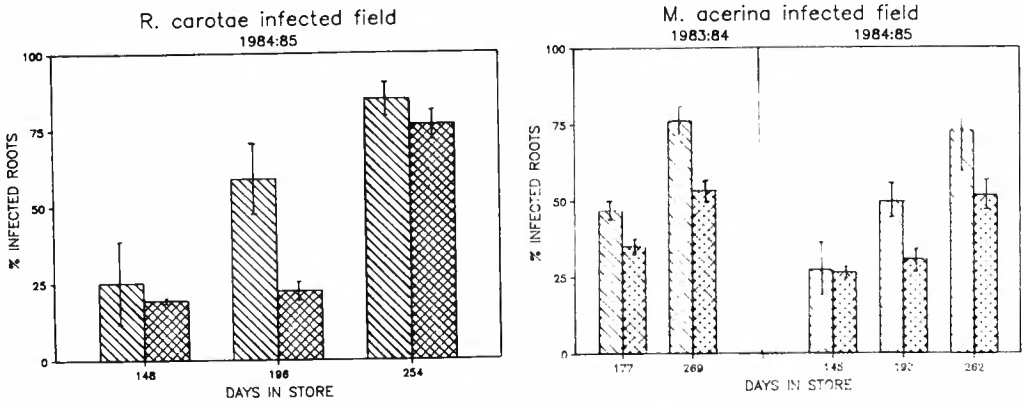

Fig. 1. Percentage of carrots with disease symptoms. Control ▨ Treated with Trichoderma harzianum P 1 ▩. Standard error of mean value is indicated by vertical lines

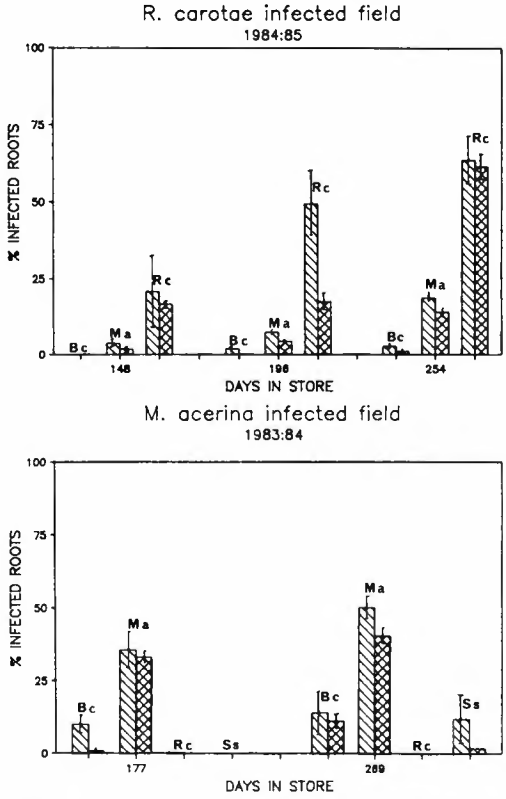

Fig. 2. Percentage of carrots with visible disease symptoms of *Botrytis cinerea* (B c), *Mycocentrospora acerina* (M a), *Rhizoctonia carotae* (R c) or *Sclerotinia sclerotiorum* (S s) after different times in cold storage.Control ▨. Treated with *Trichoderma harzianum* P 1 ▩. Standard error of mean value is indicated by vertical lines

taken into consideration when *Trichoderma* is used as a biocontrol agent.

The viable counts (incubated at 4°C) of bacteria and fungi on the carrots after storage are given in Table 1. A slight increase in the number of microorganisms during storage was seen, but there was no significant effect of the *Trichoderma* treatment on the bacterial number. *T. harzianum* was always present after isolation from the treated carrots in 1983/84 and 1984/85. Because of its high antagonistic ability and rapid growth, the *Trichoderma* soon grew over the other fungi such that the total number of these fungi on the plates was difficult to determine. Isolates of *T. harzianum* and *T. viride* were sometimes recovered from the untreated carrots. Similar results were seen in incubation at 20°C, except for a slightly higher amount of bacteria and fungi. The microbial flora was similar in 1985/86 except that no inoculated *T. harzianum* was isolated at 4°C due to its inability to grow below 5°C.

No effect on the number of anaerobic bacteria was seen after the *Trichoderma* treatment and the number increased from 10^2 to 10^6 during the storage experiment at both incubation temperatures.

This experiment indicates that biological control of storage rots on cold-stored vegetables is possible. By further selection of isolates, antagonistic at low temperatures, from naturally occurring

Table 1. Aerobic bacteria and fungi isolated from carrots harvested in 1984 and stored in a cold room. The carrots were either stored untreated (control) or treated with a conidial spore suspension of *Trichoderma harzianum*. Incubation temperature 4°C

	$\log_{10}$ organisms/g of root							
	Mycocentrospora acerina infected field				*Rhizoctonia carotae* infected field			
	Control		*T. harzianum*		Control		*T. harzianum*	
Days	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
0	4.4	3.3	4.5	3.3	5.5	3.3	5.5	3.3
145	6.9	3.8	5.8	3.6	5.9	3.6	6.0	3.7
190	6.9	4.2	6.0	3.6	6.3	3.7	6.4	3.6
255	6.8	3.7	6.6	3.6	5.7	3.7	6.0	3.7

isolates, from mutated isolates or from isolates made after genetic manipulation, it should be possible to develop this method further as a post-harvest treatment of coldstored crops.

ACKNOWLEDGEMENTS

The author thanks Sylvia Bredholt for technical assistance, the Department of Vegetable Crops for storage facilities and the Agricultural Research Council of Norway for financial support.

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OXIDIZED FLAVOUR AND XANTHINE OXIDASE IN MILK

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Astrup, H. N. 1989. Oxidized flavour and xanthine oxidase in milk. Norwegian Journal of Agricultural Sciences 3:163-171. ISSN 0801-5341.

Xanthine oxidase and oxidized flavour in milk were influenced by the physical and chemical conditions of the milk, and also change with feeding and animal factors .

Key words: Oxidation milk xanthine oxidase.

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An oxidized flavour in milk is easily recognizable. Flavour defects vary greatly in intensity, but it is debatable whether the pro- and anti-oxidative compounds in the milk, like copper and vitamin E, are wholly responsible for these differences. Enzymes or enzyme - like factors are thought to catalyse the oxidation of milk fat; e.g. copper-proteins (Jennes & Patton 1959), copper-lipoproteins (Tarassuk & Koops 1960) or the enzyme xanthine oxidase (Aurand et al. 1959). Enzymes, however, might not appear in the role of lipoxidases; they may enter secondary processes, producing or removing the substrates, products, catalysts and inhibitors of the oxidation. The role of xanthine oxidase as a pro-oxidant in milk has been debated and questioned since the early sixties (Smith & Dunkley 1962, Astrup 1963a, 1963b, Della Monica, Calhoun, Lawson, Craig & Aceto 1965) and the discussion continues to the present day (Kiermeier & Grassmann 1967, Aurand 1977, Hill et al. 1977, Allen & Wrieden 1982).

The toxicity of oxygen was realized recently when pure oxygen gas was applied in hospitals, and it has been found that the intermediates of oxygen reduction may be harmful to tissue and single cells (Halliwell & Gutteridge 1985). The bacteriostatic action of the white cell may be an oxidation. It has long been recognized that bacteriostatic effects are present in milk, oxidation enzymes possibly being involved (Nilsson 1957, Bjørk 1978, Hill 1979, Kankare & Antila 1982, Sieber 1983, Mattila 1985, Allan & Joseph 1985). The enzymes of interest are xanthine oxidase, peroxidase, catalase and superoxide dismutase. Xanthine oxidase activity varies in cows' milk (Rajan et al. 1962).

Oxygen may have harmful effects on tissue cells in several diseases. Also, the oxidation of tissue lipids may, in theory, take place. Oster (1971) proposed that lipid oxidation occurs in the arterial wall and that this leads to sclerotic changes. He also believes that particles of xanthine oxidase deriving from the drinking and digesting of homogenized milk are harmful. Xanthine oxidase was given a role as pro- oxidant.

This paper reports on a study of xanthine oxidase activity, and on how it compares with oxidized flavour develop-

ment in milk.

Xanthine oxidase

The xanthine oxidase enzyme occurs in a great variety of species and tissues and varies in properties depending on the sources. It oxidizes hypoxanthine and xanthine and it probably functions in the degradation of nucleic acids. Aldehydes are oxidized to acids. The electron acceptors of the reactions also show low specificity, and are as different as molecular oxygen and methylene blue.

Three enzymes seem to interconvert in the xanthine oxidase system (Coughlan 1980): aldehyde oxidase (EC 1231) xanthine oxidase (EC 1232) and xanthine dehydrogenase (EC 121.37). The by-product of oxidation is hydrogen peroxide, but small amounts of superoxide radical may also appear (Fredovich 1970).

Determination of xanthine oxidase activity

The xanthine oxidase enzyme activity was determined at 20°C with formaldehyde as the reducing substrate and methylene blue as oxidant and indicator.

Two 16 x 160 mm test tubes were given 0.4 ml reagent mixture with 0.5% formaldehyde and 0.4% methylene blue in distilled water, then 10 ml of milk was added to each of the two tubes, to make duplicates. The negative measure of enzyme activity was the time of bleaching in hours. An increase in concentration from 0.5% to 4% formaldehyde in the reagent decreased the time of discoloration proportionally. Raising the methylene blue level in the reagent from 0.1 to 0.4% produced a linear increase in time of discoloration.

All milks were subjected to temperatures of 15°C or lower in order to activate the enzyme.

Oxidized flavour in milk

Oxidized or metallic flavour in milk is the physiological response to small amounts of lipid oxidation products of unsaturated milk lipids. The principle compound responsible appears to be a monounsaturated volatile ketone, oct-1-en-3- one (Stark & Forss 1962). This compound is not readily oxidized further in milk products.

The oxidized flavour is reinforced by trace amounts of copper salts in so-called spontaneous milks. Oxygen can be removed by various methods - by vacuuming, by nitrogen flushing (Astrup 1963a), or by adding a culture of reducing lactic acid bacteria (Solberg et al. 1962). Physical treatments of milk, such as agitation, homogenization or even air bubbling help against the defect (Thurston et al. 1936, Tracy et al. 1933). These effects would indicate the involvement of membrane material in the oxidation mechanism.

The animals, individual and physiological states are factors to be considered, as is the kind of feed given (Astrup 1966d). The feeding of straw, hay and concentrates increased the defect, while fresh grass and silage decreased it, as did the supplement of rape seed meals, vitamin E and anti-oxidants. Protected vegetable oils increased the defect (Astrup 1972), while hydrogenated marine oil supplements appeared to be beneficial (Sundstöl 1974).

Oxidized flavour assay

Samples taken from the farmers, milk deliveries at the dairy and from the milk machine buckets in the barn were treated similarly. Care was taken to avoid contamination with copper. The samples were shaken up, and 20 ml milk was poured into 16 x 160 mm test tubes. The racks containing the tubes were left for 48 hours at 5°C before tasting.

The defect was shown to have increased from the first to the second day in the cold room. Tubes were selected because there was a stronger flavour development in the tubes than in wider vessels (Astrup 1963a).

The milk was judged by the expert taster and Milk Board consultant Hans

Jetlund, whose scorings had previously been shown to be exceptionally accurate and repeatable (Astrup 1962). Common procedures were followed (Jetlund 1954, Oterholm 1960). The scale of scorings, however, was extended to 6 steps, from zero to 6 points with increasing defect.

The samples were left for 20 min in a water bath at 25°C, and then tasted using a rustproof tablespoon.

Oxidized flavour problems were particularly severe following a drought in summer 1959.

RESULTS

Oxidized flavour and xanthine oxidase in milk samples

Milk samples from 101 cows were analysed in March 1960 and oxidized flavours were noted in 35 of them. The xanthine oxidase activity appeared the same wether with or without an oxidized flavour defect. Time of discoloration was 6.7 and 6.8 h in the two groups. However, a trend within those milks with a defect ($r = -0.24$) was stronger and statistically significant in 55 samples investigated in March 1963 ($r = -0.52$, Fig. 1). In February 1965, milk from 22 different farms in the University area was analysed twice, and once again the correlation appeared significant ($r = -0.53$ and $r = -0.55$, Astrup 1966b).

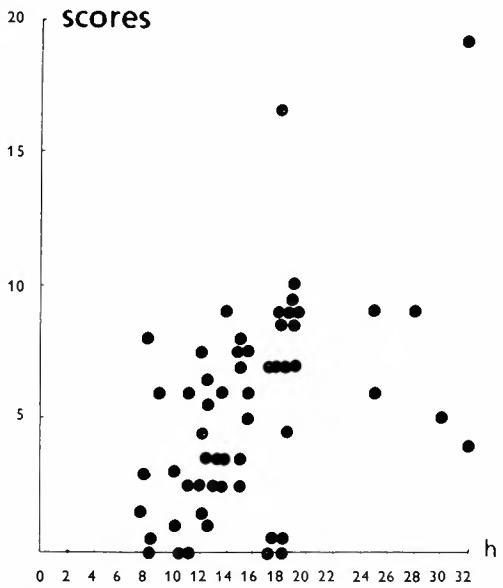

Fig. 1. Oxidized flavour scores and time of discoloration in individual cow's milk

Oxidized flavour, xanthine oxidase and pH in milk

The effect of changing the pH by adding acids or alkali to milk was investigated. The oxidized flavour of milk decreased from low to high values of pH (Table 1).

Time of methylene blue discoloration was followed in samples taken from the dairy storage containers on days 17 and 18 March 1960 (Fig. 2). Raising the pH values increased the enzyme activity. Individual samples from the barn obtained on 31 March gave the same result. Raising pH values in cows' samples of milk increased xanthine oxidase activity (Table 2). Thus, when pH values were raised, the oxidized flavour in milk decreased and the xanthine oxidase activity increased.

Temperature of reactions, oxidized flavour and xanthine oxidase

Oxidized flavour was judged in February 1960 in 72 samples from the cows in the herd. The milk had been stored at 5, 10 and 15°C and the flavour defect occurred in 47, 23 and 4 of the samples respectively. The points of defect were 57, 17 and 3 (Fig. 3).

The experiment was repeated 2 weeks later, when 0°C storage was also included. Of 77 milks, 41, 32, 10 and 2 milk samples had the defect, while the points of defect were 28, 19, 5 and 1, respectively.

In February 1963 collected samples from the dairy both behaved similarly with respect to enzyme activity. Milk at 20, 15 and 10°C was bleached after 4. 5, 13 and 20 h (Fig. 3).

Raised temperatures increased en-

Table 1. Effect of changing pH on oxidized flavour

Date		Milk from	No. of samples	Acid	pH	Oxidized flavour Control	Treatment
April	60	barn	71	citric	5.9	82	154
»	»	dairy	12	lactic	6.5	9	20
»	»	»	12	citric	6.4	9	26
»	»	»	12	HCl	6.5	9	20
»	»	»	12	lactic*	6.5	24	38
»	»	»	12	citric*	6.4	24	34
»	»	»	12	HCl*	6.5	24	37
April	63	barn	43	citric	6.4	32	49
»	»	»	43	NaOH	7.0	32	15

*added 0.1 mg Cu per litre milk

zyme activity, but depressed the oxidized flavour in milk.

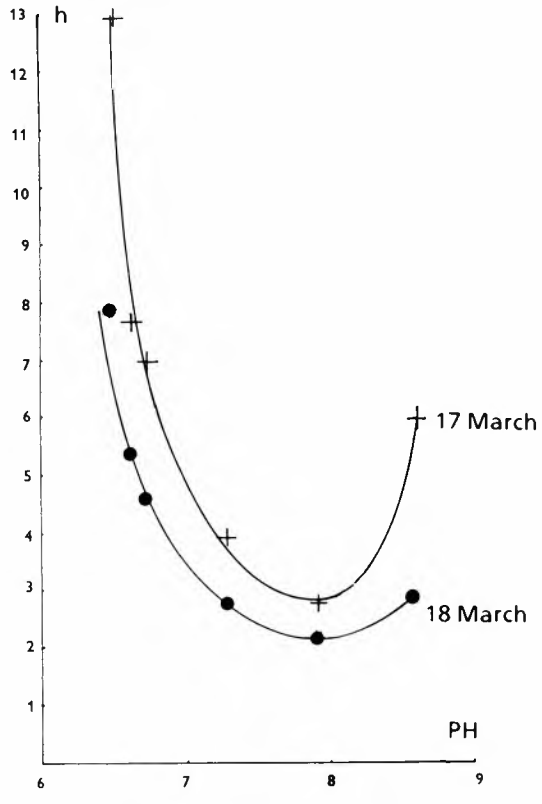

Fig. 2. The pH-dependency of discoloration in milk

Table 2. Effect of increasing pH on xanthine oxidase activity in cows milk

Cow	Bleaching time, h pH 6,8	pH 8,0
1611	4.3	2.0
93	4.4	2.5
1603	5.1	2.0
1633	5.1	2.3
1585	5.0	2.3
1634	6.1	2.8
1630	7.0	3.6
96	8.3	3.9
80	8.3	3.9
1627	9.0	5.0
1622	9.0	5.2
1626	9.0	6.0
Mean	6.7	3.5

Oxidized flavour, xanthine oxidase and additives in the milk

When added to the milk also compounds acting as substrates, catalysts or inhibitors must affect both reactions simultaneously if there are to be causative relations between them.

In Table 3 it is shown that adding compounds produced less oxidized flavour in milk, while in Table 4 adding compounds produced more oxidized flavour in milk. With the exception of hydrogen peroxide, pteridylaldehyde and EDTA, other additives were reduc-

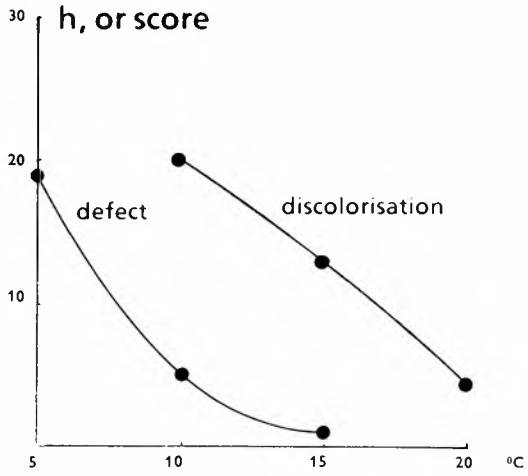

Fig. 3. The temperature dependency of oxidized flavour and discoloration in milk

ing compounds. Vitamin C and hydrogen peroxide turned pro-oxidative with increasing Cu contents. Formaldehyde and hypoxanthine are reducing compounds and also substrates to xanthine oxidase; they produced less oxidized flavour. While pteridylaldehyde is an inhibitor to xanthine oxidase (Fig. 4), it produced more oxidized flavour when added to the milk. All these effects might indicate an anti-oxidative or defect- reducing role of the enzyme in milk.

Xanthine oxidase and additives

Mixed milk samples from the dairy and from the farm were tested. As expected, the substrate additions of formaldehyde and hypoxanthine reduced the time of bleaching. But ascorbic acid and cystein also shortened the time of discoloration. Their effects were in area 10-70 ppm, however, less dependent upon their concentration, thus indicating a role different from that of a substrate. Hydrogen peroxide and copper were found to be inhibiting, depending upon their concentration. Inhibition was noted from 0.1 ppm Cu or from 6 ppm H_2O_2 and upwards. Pteridylaldehyde was inhibiting from 1 ppm, and inhibition increased with level of addition (Fig. 4).

Feed and animal factors, oxidized flavour and xanthine oxidase

The two milk parameters are compared in two experiments in Table 5. One group of six animals and another of 12 animals were fed 1 kg of various seeds along with the concentrate in a latin square change-over design. The results show that rape seed or rape seed meal gave the least oxidized flavour defect,

Table 3. Additives decreasing milk oxidized flavour defect scores

Milk from	No. of samples	Additive ppm	Control defect score	Treatment defect score
Barn	11	EDTA,100	74	18
	11	Cystein, 40	74	38
Dairy	33	do, 20	75	38
Barn	11	Vit C,100	50	35
	11	do, 50	33	5
	11	do, 25	100	42
	22	H_2O_2, 4	125	136
	22	do, 8	125	50
	22	do, 15	87	42
	11	do, 30	50	1
	11	do, 60	74	2
	64	HCHO, 35	67	37
	67	do, 35	79	63
Dairy	45	Hypoxanthine, 20	33	25

Table 4. Additives increasing milk oxidized flavour defect scores

Milk from	No. of samples	Additive ppm	Control defect score	Treatment defect score
Barn	11	Cu++, 0.1	74	164
	11	Fe++, 0.1	74	96
Dairy	46	Vit C, 50*	28	44
	45	H_2O_2, 20*	29	64
	43	pteridylald, 20	32	67
Barn	67	do, 20	79	100
Dairy	45	do, 20	29	39

*milks added 0.4 ppm Cu++

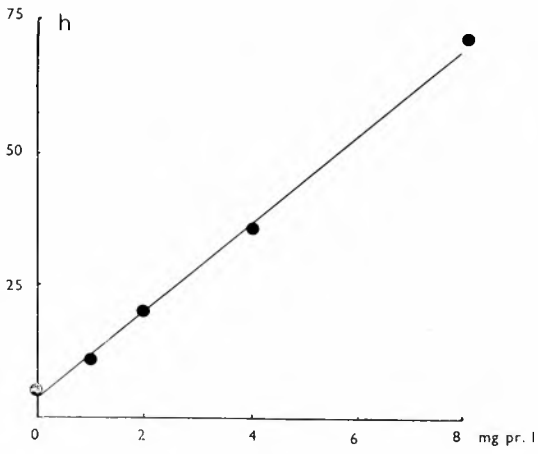

Fig. 4. Increase of discoloration time adding pteridylaldehyde to milk

Table 5. Oxidized flavour and xanthine oxidase in milk affected by thyroxine, age and state of lactation

Condition	Oxidized flavour, points	Methylene blue reduction, h
Control	129	5.9
Thyroxine	163	8.5
Early lactation	153	7.8
Late lactation	122	5.4
Young animals	170	8.6
Older animals	104	5.0

and also the highest xanthine oxidase activity (Astrup 1966a).

In Table 6 the two milk parameters are compared within a change-over design with 12 animals; treatments are with thyroxine and with blocks composed of early and late lactation or young and older animals (Astrup 1966c).

Thyroxine treatment, young animals and early lactation all reflected the highest oxidized flavour but also the lowest of xanthine oxidase activity.

DISCUSSION

If xanthine oxidase is significant for the development of oxidized flavour, then oxidized flavour and enzyme activity

Table 6. Oxidized flavour and xanthine oxidase in milk affected by feeding rape seeds or meal

Treatment	Oxidized flavour, points	Methylene blue reduction, h
Exp. I		
Linseed	94	4.1
Soybean	55	5.5
Rape seed	27	3.8
Exp. II		
Soybean	77	6.5
Rape seed	37	5.1
Rape meal	37	5.4

must be positively correlated in arbitrarily chosen samples of milk, and if even distribution of the scores is to be obtained. (This requirement was fulfilled with sensitive methods of analysis and of scorings by the judge.) The two parameters turned out to be negatively correlated.

Further more, the characteristic properties of enzymes, such as their pH and temperature dependency, must be reflected in oxidative flavour development. The two parameters, however, turned out to be negatively correlated.

Further, compounds in or added to the milk, acting on the enzyme, also ought to affect the oxidized flavour. Again the two parameters generally tended to be negatively related.

Finally, feed and animal factors also produced negative correlation between the milk oxidized flavour and xanthine oxidase activity.

Xanthine oxidase in milk does not fulfil the expectations of its being the dominant pro-oxidant in milk. The enzyme properties seen here indicate an anti-oxidative role in the development of the defect. The less enzyme activity, the more the oxidized flavour was detected in the milk.

The behaviour of oxidized flavour in milk to hydrogen peroxide additions, when Cu was not added, may appear contradictory. However, this can be accounted for if it is realized that oxidizing compounds may have an effect on the catalytic property of Cu.

Experience with the milk, also in the present study, showed the copper catalysis in milk to be fundamental. In the milk lipids chain reactions may be short, and initiation by copper particularly important.

In the so-called Fenton reaction, initiation by free radicals takes place when copper or iron are present in their reduced state. All the reducing compounds in the milk, however, appeared in this study to be anti-oxidative. This property is accordingly stronger than their effect on the copper catalysis.

The anti-oxidative property of reducing compounds in milk is hardly explainable by way of free radical scavenging. The effect may be that of lowering the milk's oxygen content. Ascorbic acid and SH-containing compounds likely react spontaneously with oxygen. Xanthine and hypoxanthine are also reductants, and remove oxygen through enzymatic oxiddation catalysed by xanthine oxidase.

In theory, a dualistic concept of reducting compounds action may allow for xanthine oxidase to bring about one or the other situation, depending on the conditions. Such thinking may be in line with the suggested rH dependency of oxidation (Greenbank 1940). However, our study indicates that in milk the enzyme usually acts to prevent lipid oxidation.

Xanthine and hypoxanthine are the possible substrates and derive from degradation of body and rumen cells and bacterias in milk. Nilsson (1957) finds that both enzyme and substrate levels increase in mastirrtis. The prevention of oxidized flavour by adding reducing lactic acid bacteria may depend upon xanthine oxidase activity (Solberg et al. 1962).

Obviously, the study of xanthine oxidase and its role in lipid oxidation is difficult, since results and conclusions vary. Model experiments may give artifacts. Adding enzymes or protein preparations may introduce excess copper, as suggested by Smith & Dunkley (1962).

Difficulties may also arise in the interpretation of studies where lipid substrates have been introduced into the milk. Oxygen consumption or rH values are explicits of xanthine oxidase catalysed oxidations and of lipid autoxidation. Care must be taken to ensure independency of the variables.

SUMMARY

Xanthine oxidase, and oxidized flavour was assayed in milk samples from the University Herd and District Dairy. The effects on milk of different storage temperatures, of pH, of additions, of feeding oil seed meals or thyroxine, and of age or state of lactation of the cows have been studied.

All treatments affected the results. The two parameters showed negative correlation, and indicated an anti-oxidant rather than a pro-oxidant role of xanthine oxidase in milk.

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THE EFFECT OF LIME ON MAIZE AND GROUNDNUT YIELDS IN THE HIGH RAINFALL AREAS OF ZAMBIA

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Tveitnes, S. & H. Svads, 1989. The effect of lime on maize and groundnut yields in the high rainfall areas of Zambia. Norwegian Journal of Agricultural Sciences 3:173-180. ISSN 0801-5341.

In a long-term lime trial in the high rainfall areas of Zambia the effects of varying amounts of lime on maize and groundnut yields were mostly insignificant in the first four seasons. In the 5th to 9th seasons the highest maize crop yields were obtained from an initial application of 4000 kg of lime per hectare. In the 12th and 14th seasons 500 kg of lime per hectare annually was the highest yielding treatment. On average the treatments of 4000 kg of lime initially and 500 kg of lime annually equally improved maize crop yields. In groundnuts, crop yield response was highest due to the residual effect of lime that had been applied initially, while in the 11th and the 13th seasons annual applications of 500 kg of lime per hectare had the best yield. On average residual effects of 4000 kg and 2000 kg of lime per hectare increased the yields of groundnuts but not significantly more than 500 kg applied annually.

Key words: groundnuts, high rainfall area, lime, maize, Zambia.

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Large areas of agricultural land in the high rainfall areas of Zambia are dominated by soils derived from non-basic rocks which can be dated back to the Precambrian, more than 900 million years ago.

These soils are weathered and strongly leached, and they do not readily retain nutrients due to their low cation exchange capacity. The main clay mineral is kaolinite. Most of the soil is acidic, with a pH around 4.5 or below, often associated with a rather high level of free aluminium ions in the soil, sometimes also manganese. These elements can cause toxic conditions in the plants, thus reducing growth. Cultivated plants have different levels of tolerance to acidity. Acidity trials have shown that yields of maize are reduced to practically nil under high acid conditions, and that groundnuts are more susceptible to acidity than maize.

The site of the present lime trial is at Misamfu Regional Research Station, north of Kasama. The approximate location is 10°10'S and 31°10'E, at an altitude of 1380 m above sea level. The climate is strongly seasonal with a wet season from November to April and a dry season from May to October.

The object of the experiment was to determine the effect of lime on acid soils bearing maize and groundnuts, and to determine the optimum rate of lime application.

MATERIALS AND METHODS

The experimental design was randomized block with 4 replicates and 6 treatments; 2 crops (maize and groundnuts). The plot size was 12.2 x 9.1 m^2. For maize, row and plant spacing was 91 cm and 25 cm, respectively, and for groundnuts 86 cm and 10 cm. Paths (1.8 m in width) were made between blocks in each replicate. The total trial site was 0.534 ha.

In the first 5 years the two crops were grown in rotation. In 1976-77 only groundnuts were harvested, while in 1977-78 and 1978-79 maize was grown in the field. In 1979-80 groundnuts were grown every other year, rotating with maize until 1984-85.

The following amounts of base fertilizers were applied to the plots and hoed in before sowing:

N	100	kg/ha in ammonium nitrate, and
	21	kg/ha in sulphate of ammonia, of which 6 kg/ha was top-dressed.
P	26.2	kg/ha in triple super phosphate
K	41.3	kg/ha in sulphate of potash
S	16	kg/ha in sulphate of potash, and
	24	kg/ha in sulphate of ammonia
B	2	kg/ha in solubor

The base fertilizer was applied every year.

Lime treatments.

1. No lime (control)
2. 500 kg/ha
3. 500 kg/ha (Applied every year. In tables referred to as 500+)
4. 1000 kg/ha
5. 2000 kg/ha
6. 4000 kg/ha

Treatments 2, 4, 5 and 6 were applied in order to measure residual lime effects. Maize variety SR 52 was sown on flat land, while the groundnut variety Makulu Red was sown on ridges. Ploughing was carried out in alternate directions in alternate years; a two-way plough was used to avoid cumulative physical movement of the soil.

Soil samples were collected from the 0-15 cm and 20-40 cm soil layers before the application of fertilizer and lime.

Dates of operations over years.

Ploughing and discing	10	August	-	6	December
Application of base fertilizer to maize	6	November	-	7	December
Application of base fertilizer to groundnuts	3	December	-	8	December
Sowing of maize	20	November	-	19	December
Sowing of groundnuts	4	December	-	22	December
Application of top-dressing	25	December	-	22	January
Harvesting of maize	8	April	-	8	June
Harvesting of groundnuts	9	May	-	2	June

RESULTS

Maize

In the first season 1971-72, the 1000 kg per hectare lime treatment failed to outyield the control, but sigificantly outyielded both the 500 kg and the 2000 kg of lime per hectare treatments; the yield increases were 948 kg and 946 kg per hectare. There was no significant difference between the 500 kg and 2000 kg per hectare lime treatments, nor between the control and the 4000 kg per hectare of lime.

During the next three seasons, none of the residual lime treatments nor the 500 kg lime per hectare applied annually had any significant effect on the crop yield as compared with the control.

It was not until the 5th experimental year, that significant and positive responses to liming were obtained in maize. All treatments applied initially and 500 kg per hectare applied annually outyielded the treatment where 500 kg per hectare lime was given at the start of the experiment only. Otherwise there were no significant differences.

Table 1. Yields of maize during the experimental period. Kg ha^{-1}

Season	Lime, tons ha^{-1} 0	500	500 +*	1000	2000	4000	Mean	$LSD_{5\%}$
1971-72	4717	4108	4108	5054	4106	4401	4415	n.s.
1972-73	4183	3579	4253	3931	3528	4057	3922	n.s.
1973-74	4103	3699	3554	4480	3800	3775	3902	n.s.
1974-75	2430	2768	2944	2365	2768	3196	2730	n.s.
1975-76	4406	3876	4557	4580	4505	4858	4464	434
1977-78	260	369	693	592	769	895	596	215
1978-79	2235	2635	3219	2697	3397	3372	2926	n.s.
1980-81	1840	2192	3441	2559	3205	3538	2796	586
1982-83	1506	1496	4423	1796	3241	4107	2762	1103
1984-85	478	294	3564	678	1470	2642	1521	650
Mean	2607	2502	3476	2873	3079	3484	3003	530

* Applied every year

In 1977-78, the yield responses to the residual effect of 2000 kg and 4000 kg and to the effect of 500 kg per hectare lime applied annually were significant. A tendency towards a residual effect was also demonstrated where 1000 kg and 500 kg of lime per hectare were applied initially. The overall crop yield in the trial was very poor. The plants were reported to be mostly stunted and yellowish in colour, probably due to a lack of nitrogen, as no fertilizer was applied.

In 1978-79, there was a tendency to better yield where the largest amounts of lime were applied, but the differences were insignificant.

In 1980-81 rat damage was substantial. Crop growth was quite vigorous, especially after the ammonium nitrate dressing. The occurrence of stalkborer was minimal. As shown in Table 1, 4000 kg per hectare of lime gave the highest mean yield of 3538 kg maize per hectare, followed by the treatment where 500 kg was applied annually.

Liming with 500 kg per hectare every year gave the highest yield of maize in 1982-83 and in 1984-85. The 4000 kg and 2000 kg treatments applied at the start of the experiment also increased the yield significantly, while the effects of 500 kg and 1000 kg per hectare at the start of the experiment were insignificant.

Groundnuts

In the first season, the treatment 4000 kg per hectare of lime just failed to outyield the treatment 2000 kg, but outyielded all the others. The following year (1972-1973), the differences between crop yields were small. However, 1000 kg of lime was the highest yielding. The control plots had the lowest yield.

In the third season (1973 - 1974), the application of 2000 kg of lime per hectare (residual effect) resulted in the highest mean yield; 1328 kg groundnut kernels per hectare. All the treatments outyielded the control. The 2000 kg lime application also outyielded the 500 kg, 1000 kg and 4000 kg per hectare treatments.

In 1974-75, the plots that received 500 kg per hectare of lime annually outyielded all the other treatments with a yield of 1071 kg kernels per hectare. Application of 4000 kg of lime per hectare outyielded the other treatments, and the control.

Table 2. Yields of shelled groundnuts during the experimental period. Kg ha^{-1}

Season	Lime, kg ha^{-1} 0	500	500+*	1000	2000	4000	Mean	$LSD_{5\%}$
1971-72	736	1120	948	1016	1196	1308	1054	n.s.
1972-73	1108	1398	1253	1512	1416	1272	1327	n.s.
1973-74	636	1072	972	1036	1328	1100	1024	n.s.
1974-75	880	816	1071	843	871	989	912	n.s.
1975-76	523	839	579	825	1020	916	784	124
1976-77	281	348	520	446	515	526	439	60
1979-80	732	909	1611	1294	1748	1741	1339	88
1981-82	276	380	1407	639	1018	1384	851	194
1983-84	116	98	895	369	375	643	416	194
Mean	588	775	1028	886	1054	1098	905	200

* Applied every year

In the fifth season (1975-76), the highest yielding treatment was 2000 kg lime per hectare. Also the 4000 kg, 1000 kg and 500 kg of lime outyielded the control plots.

In 1976-77, the highest crop yield was obtained from the 4000 kg of lime per hectare treatment, although the 500 kg applied every year and the 2000 kg treatment both responded well. These yields were significantly higher than those of treatments nil and 500 kg of lime per hectare.

Groundnuts were then not grown in the field trial until 1979-80 when the yield response to the highest amounts of lime was considerable. In the final years (1981-82 and 1983-84), plots having received 500 kg per hectare of lime annually turned out to be the highest yielding.

In general, emergence of the crop was quite satisfactory except on the control plots. The crop was vigorous in growth and free of any disease; the only pest viewed, was the leaf-eating caterpillars, were taken care of by spraying with DDT 75%. However, it is noticeable that plots with zero and those with 500 kg per hectare of lime had revealed plant pops within their pods at the time of shelling.

As can be seen from Table 2, in 1979-80 treatment 2000 kg lime per hectare gave the highest yield of 1748 kg kernels per hectare, followed by treatment 4000 kg per hectare. Application of 500 kg of lime annually also gave a significant yield response, although less than the residual effect of the highest lime applications.

Soils and soil analysis

The soil at the trial site is Misamfu Sandy Soil, which is a deep well drained sandy clay loam with a sandy loam surface soil. The soil reaction is very acid. According to the USDA Soil Taxonomy System it is classified as Typic Haplustox. The A-horizon (0-11 cm) is dark yellowish brown sandy loam, moderate fine subangular blocky, with many micro and fine pores. The A_3-horizon (11-37 cm) is yellowish red sandy loam (van Sleen, 1976).

Soil samples were collected from the field trial in 1971,1972, 1973 and 1981. Samples from 1971 just before liming indicate that soil pH measured in $CaCl_2$ was 4.5 in both the 0-9 cm and the 9-18 cm soil horizons. The following year, application of 4000 kg and 2000 kg lime per hectare increased pH in the top 0-6 cm to 5.1 and 4.7 respectively. Otherwise, negligible changes in pH took

Table 3. Soil pH analysis

	Soil Depth	Lime, tons ha^{-1} 0	500	500 + *	1000	2000	4000
pH ($CaCl_2$)							
1972	0-6	4.5	4.4	4.5	4.5	4.7	4.4
	12-15	4.6	4.4	4.4	4.4	4.4	4.4
1975	0-15	4.7	4.7	4.6	4.6	4.9	5.1
1981	0-15	4.2	4.1	4.7	4.2	4.3	4.7
	20-40	4.3	4.2	4.3	4.2	4.3	4.6
pH (H_2O)							
1981	0-15	4.8	4.6	5.3	4.7	4.9	5.3
	20-40	4.9	4.8	4.9	4.7	5.0	5.1

* Applied every year

place. The subsoil pH was not changed. Analyses carried out in 1975 indicated corresponding results. In 1981, pH dropped in all plots; it was only 4.2 in the control plots, and 4.7 in plots treated with 4000 kg lime per hectare ten years earlier and in plots with application of 500 kg lime per hectare annually.

While in 1971-72 no significant differences in the content of calcium, magnesium or potassium was found, soil analysis in 1973 showed that liming increased the levels of calcium, CEC and base saturation (BS %) significantly.

As shown in Table 5, the aluminium content of leaves, being higher than 0.70 m.e./100 g in unlimed and weakly limed soil, was reduced to 0.22 m.e./100 g as the residual effect of 4000 kg lime per hectare at the start of the experiment.

Exchangeable calcium and magnesium also increased when the highest

Table 4. Chemical soil analyses in the 1972-73 season. Cations and CEC in m.e./100 g soil

	Depth	Lime, tons ha^{-1} Control	500	500 + *	1000	2000	4000	Mean	$LSD_{5\%}$
Ca	0-6	0.42	0.70	0.51	0.68	0.86	1.53	0.78	0.47
	12-15	0.11	0.22	0.22	0.14	0.28	0.31	0.21	0.10
Mg	0-6	0.35	0.34	0.28	0.33	0.30	0.38	0.33	n.s.
	12-15	0.25	0.22	0.26	0.23	0.18	0.19	0.22	n.s.
K	0-6	0.12	0.09	0.07	0.09	0.08	0.08	0.09	0.02
	12-15	0.11	0.07	0.07	0.06	0.05	0.05	0.07	n.s.
CEC	0-6	3.65	3.82	3.67	3.72	4.05	4.87	3.96	0.80
	12-15	2.50	2.62	2.54	2.74	2.70	2.80	2.65	n.s.
BS %	0-6	24.1	29.4	22.8	28.2	30.9	42.7	29.7	12.5
	12-15	18.8	19.4	21.5	15.7	19.7	19.9	19.2	n.s.

* Applied every year

Table 5. Chemical soil analysis in 1981. Samples from the 0 - 15 cm soil horizon. Exchangeable cations and CEC in m.e./100 g soil. Organic C in g/100 g soil. Avail. P and Mn in mg kg^{-1}.

	Lime, kg ha^{-1}							
	0	500	500+*	1000	2000	4000	Mean	$LSD_{5\%}$
Exch. Ca	0.42	0.30	1.07	0.40	0.70	1.36	0.71	0.23
» Mg	0.07	0.13	0.16	0.09	0.10	0.14	0.12	n.s.
» K	0.05	0.05	0.06	0.05	0.05	0.04	0.05	n.s.
» Na	0.05	0.05	0.06	0.05	0.04	0.05	0.05	n.s.
» Al	0.72	0.77	0.28	0.83	0.61	0.22	0.57	n.s.
CEC	4.38	4.37	4.60	4.25	5.15	4.70	4.41	n.s.
Organic C	0.83	0.65	0.65	0.64	0.82	0.80	0.73	0.16
Avail. P	32.8	30.8	27.4	29.9	28.7	27.8	29.6	3.91
Mn	4.2	4.3	2.6	4.3	3.5	2.5	3.6	n.s.
BS %	13	13	30	14	19	34	21	2.2

* Applied every year

amounts of lime were applied. Leaf content of other cations, organic carbon and nitrogen were not affected by liming.

Leaf analysis

In maize the 5th leaf from the top (flag leaf no. 1) was collected at tasselling, altogether 10 leaves from each plot. Samples of groundnut leaves from each plot were also analysed. As there were no significant variations between treatments, the leaf analysis data are presented as means of treatments. Leaf samples were analysed in the seasons 1971-72, 1972-73 and 1973-74.

Analysis of manganese in maize leaves was carried out in 1972 and showed a mean content of 5.82 mg kg^{-1}. The content of zinc in maize leaves was analysed in 1974, when the mean was 50.2 mg kg^{-1}. Neither manganese nor zinc values varied significantly between treatments.

DISCUSSION

In the first four years of the experimental period, liming did not improve the yield of either maize or groundnuts significantly. This is in accordance with a number of reports citing lack of response or the negative response when tropical soils are limed (Sanches 1976). Large amounts of lime per hectare may decrease the availability of phosphorus, boron, zink, manganese, etc. Liming at rates higher than necessary to neutralize the exchangeable aluminium or to eliminate manganese toxicity may cause the soil structure to deteriorate (Kamp-

Table 6. Chemical analyses of maize and groundnut leaves. Macronutrients in g kg^{-1}, B in mg kg^{-1}.

	Maize leaves			Groundnut leaves		
	1971-72	1972-73	1973-74	1971-72	1972-73	1973-74
N	22,9	19,7	28,4	-	48,8	48,2
P	3,2	3,1	3,8	3,8	3,3	2,7
K	21,8	17,0	16,0	29,4	21,0	32,4
Ca	3,9	4,4	4,8	8,1	12,7	11,6
Mg	3,1	3,1	2,0	5,6	6,8	3,4
B	6,21	7,46	-	29,9	35,6	-

rath 1971). Lime trials in Zambia have also shown that low levels of lime are required to neutralize soil acidity (Ann. Reports of the Research Branch (1963-74, Munyinda 1983).

In the fifth season, significant and positive responses were obtained due to the residual effects of liming to maize. In the following years too, a notable maize yield response was demonstrated for the residual effect of the highest amounts of lime. At this particular site, the residual lime effect is obviously long term.

In 8 years, the plots with 4000 kg per hectare at the start of the trial and those with 500 kg every year had received the same amount of lime. On average for this period the residual effect of 4000 kg lime per hectare yielded higher, but not significantly higher, than plots which received 500 kg lime per hectare annually. On unlimed plots there were eventually reduced yields throughout the experimental period, except for 1975-76 when maize yields were very high, and 1977-78 when the yields were extraordinarily low. Liming retarded maize yield reduction, and with the exception of the 1977-78 season the yields were on an acceptable level.

In groundnuts there was no significant yield response from liming the first four years, although there was a tendency for the yield of groundnuts to be higher on limed plots. In later seasons limed plots yielded better than unlimed. Annual liming with 500 kg lime per hectare yielded better but not significantly better than the residual effect of 2000 kg and 4000 kg lime per hectare.

The main reasons for the poor growth on acid soils in the tropics are aluminium and manganese toxicity and calcium and magnesium deficiency. In this trial, liming reduced the level of exchangeable aluminium and manganese, while the amount of exchangeable calcium and magnesium was increased, as was pH where the highest amounts of lime were applied. The positive effect of liming obtained in this trial is very likely a combined effect of most of these factors.

In this experiment the residual effect of 4000 kg lime per hectare increased the yields of both maize and groundnuts on average for the experimental period to about the same level as did 500 kg lime applied annually. Since heavy amounts of lime per hectare may cause reduced availability of important plant nutrients, and may even cause the soil structure to deteriorate, more frequent liming with smaller amounts per hectare each time may be preferable and should be recommended.

In the last season, yields of unlimed and weakly limed plots reached their lowest point during the whole experimental period. Plots which received 500 kg ha^{-1} every year were the highest yielding the last two seasons.

Looking at the average yields of groundnuts obtained during the whole period, the treatments 500 kg per hectare every year and 2000 and 4000 kg at the start of the experiment were the best. Average yields obtained were 1028-1098 kg per hectare compared with 588 kg on unlimed plots.

SUMMARY

The report describes a long-term lime trial at Misamfu Regional Research Station, Northern Province of Zambia. Treatments comprised lime applied at 500 kg, 1000 kg, 2000 kg and 4000 kg per hectare at the start of the trial, 500 kg applied annually and control (without lime).

The effects of all treatments were mostly insignificant during the first four seasons in both maize and groundnuts. During the 5th to 9th seasons the highest maize crop yields were obtained from an initial lime application of 4000 kg per hectare.

In the 12th and 14th seasons 500 kg of lime per hectare annually was the highest yielding treatment. On average,

for 10 harvestings during the 14-year period, the treatments of 4000 kg of lime initially and 500 kg of lime annually equally improved the yields of maize crop.

In groundnuts the crop yield response was highest due to the residual effect of lime that had been applied initially, while in the 11th and 13th seasons annual applications of 500 kg of lime per hectare yielded best. For the experimental period as a whole, residual effects of 4000 kg and 2000 kg of lime per hectare increased the average yields of groundnuts insignificantly better than 500 kg applied annually.

Liming reduced the level of exchangeable aluminium and manganese, while the amount of exchangeable calcium and magnesium was increased, as was pH where highest amounts of lime were applied. The positive effect of liming obtained in this trial is most probably a combined effect of improved availability of plant nutrients, and a reduction in the amount of available aluminium and manganese in the soil.

ACKNOWLEDGEMENTS

The authors thank the officers and assistants at Misamfu Regional Research Station and Mount Makulu Central Research Station who ensured the satisfactory conduct and continuity of the trial over so many years. Thanks are extended to the Norwegian Agency for International Development and the Agricultural University of Norway for financial and technical support. Grateful acknowledgement is made to the Assistant Director of Research, Ministry of Agriculture and Water Development, Lusaka for permission to publish this paper.

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MAIZE YIELD RESPONSE TO NITROGEN, POTASSIUM AND SULPHUR IN FERTILIZERS UNDER CONTINUOUS CULTIVATION IN THE SOUTHERN PROVINCE OF ZAMBIA

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The linear effect of nitrogen is very variable and interacts strongly with years, while the quadratic effect is consistently negative. On average, 136 kg N per hectare gives a significantly greater yield than the lower or higher N levels. The effect of potassium is positive but not significant in any year. When phosphorus fertilizer is applied every year it significantly increases the yield. During the first three years of the experiment it was found that 11.2 kg sulphur per hectare increased the yields significantly. No yield increase is found when the nil level is changed to 22.4 kg sulphur per hectare. Leaf and soil analysis data are not clearly influenced by the fertilizer treatments. Most nutrients decrease with years, as was found especially in the topsoil during the 1966-72 period.

Key words: Continuous cropping, maize, nitrogen, phosphorus, potassium, sulphur, Zambia

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In the first recorded field experiments in Zambia (McEwan 1932) only phosphatic fertilizers were tested; nitrogen fertilizers were not included. The first response to nitrogen in the region was reported in Zimbabwe by Rattray (1949). In the following year Pawson (1959) reported impressive responses to sulphate of ammonia in Zambia, but indicated that phosphorus responses were mainly limited to areas in the Southern Province, with no responses to potassium. Pawson realized after a few years that the considerable yield responses obtained from sulphate of ammonia could be partly due to the sulphur content in this fertilizer. As a result, a series of experiments were carried out on the more fertile soils of Central, Southern and Eastern Provinces with the conclusion that sulphur was not deficient in Zambian soils. An experiment at Mount Makulu Central Research Station supported these conclusions.

In 1958, however, reports from the Northern Province indicated that urea did not have the anticipated beneficial effect on crop growth and yield, and a

greenhouse experiment at Mount Makulu revealed a serious sulphur deficiency in soil from the Mansa District of Luapula Province (McPhillips 1983). During the period 1960-65 an extensive programme of trials showed that in the Northern, Luapula and North Western Province, and in the Eastern and Central Provinces, large significant responses to nitrogen and sulphur could be expected in all seasons. The results of the sulphur responses have been summarized by Vogt (1965). However, no responses to either phosphorus or potassium was identified.

In the Southern Province the picture was somewhat different in that large significant responses to nitrogen and phosphorus were obtained, but not to sulphur or potassium.

As most of the trials were on virgin land it was decided to carry out longer term trials with different levels of nitrogen to ascertain the extent of the soil reserves of phosphorus and potassium in the northern and eastern parts of the country, and of potassium and sulphur in the south. The trials were initiated during the period 1965-67. Because of the problems experienced in staffing and continuity only three trials, of the original six, have survived to the present day. These are at Misamfu Regional Research Station, Kabwe Regional Research Station and Magoye Regional Research Station, sites which fortunately cover the main agro-ecological zones from north to south and are sited on agriculturally important soil series. The Magoye trial is reported here.

METHODS

The experimental site is situated on the Magoye Regional Research Station about 25 km south of Mazabuka, at latitude 16°00'S and longitude 27°43'E.

The mean annual rainfall in the research period was 800 mm in 69 days, mostly from October to April, with the highest rainfall in December. In two of the seasons (1968-69 and 1977-78) the annual rainfall exceeded 1000 mm. The lowest annual rainfall was measured in the 1969-70 and 1972-73 seasons with 546 and 561 mm, respectively.

Temperature data are not available prior to 1977, but the main temperatures for the three seasons 1977-78 to 1979-80 indicate that October was the warmest month, while the temperature decreases from January onwards.

The soil at the experimental site belongs to ferric/orthic Luvisol Soil Series (in the FAO system).

The mechanical composition was determined by the «Hydrometer» method (Table 1).

The soil was also analysed for pH and selected chemical properties, using the following analytical methods: pH was determined in 0.01 M $CaCl_2$, and organic carbon by wet digestion (the Walkley and Black method). Exchangeable calcium and magnesium were determined by atomic absorption, while potassium and sodium were measured on a flame photometer. Cation exchange capacity (CEC) was determined by leaching out of the excess ammonium acetate with alcohol, and finally leaching with a sodium chloride solution to replace ammonia. In the subsequent distillation a

Table 1. Mean particle size distribution

Clay	Silt	Fine sand	Medium fine sand	Course sand	Texture
23	19	41	14	3	SCL

boric acid indicator solution was used and titration was carried out with hydrochloric acid. Base saturation (BSP) was calculated as a sum of cations in per cent of CEC. Available phosphorus was determined by Bray's no. 1 method.

Soil samples were collected from each plot annually after land preparation from depths of 0 - 15 and 25 - 45 cm. Chemical soil data are presented in Table 8. The analyses were carried out at the soil laboratory of the Mount Makulu Central Research Station, Chilanga.

Before implementation of the long-term studies the site was subjected to a uniformity trial from which the actual location of each replicate was decided. The trial was laid out as a 3x22 confounded factorial in blocks of six plots with eight replications. This included a «dummy» treatment which was utilized from the 1975-76 season when the design became a 3x23 factorial with four replications. The design is plans 6.9 and 6.10 from Cochran and Cox (1957).

Three seeds of a single hybrid maize variety (SR 52) were sown at 23 cm stations in four row plots each row 90 cm apart. One plant was retained at each station when thinned about 2 - 3 weeks after emergence. The seed bed was treated with 2.5 % Aldrin dust as a precaution against soil-borne insect pests, and 5% granular DDT was applied to funnels of the plants at about four weeks to control stalkborer. A pre-emergence herbicide (Atrazine) was applied at 2 kg per hectare shortly after sowing, and the plots were hand weeded later in the season. The direction of ploughing was alternated in consecutive years. Initially the maize stover was ploughed in but as the trial progressed diseases (Fusarium spp. and Diplodia spp. cob rots) became more pronounced in years when the rains extended beyond crop maturity and in these years the stover was burnt.

Mean dates of operations over years

Subsoiling	17 July - 10 October
Ploughing	4 August - 11 October
Discing	5 August - 14 October
Application of basal fertilizer	11 October - 14 October
Sowing	9 November - 13 December
Thinning	30 November - 3 January
Application of top dressing	15 December - 13 January

Treatments

Nitrogen

N0	78	kg N per ha
N1	136	kg N per ha
N	190	kg N per ha

Potassium

K0	Zero	
K1	9.3 kg	K per ha from 1966-67 to 1972-73, and then
40	kg	K per ha from 1973-74 to 1980-81

Sulphur

S0	Zero from 1966-67 to 1968-69	
S1	11.2	kg S per ha from 1966-67 to 1980-81
S2	22.4	kg S per ha from 1969-70 to 1980-81 (replaced S0)

All the nitrogen plots received 44.8 kg N in ammonium nitrate per ha sidebanded before planting, followed by top dressing of the remainder some three weeks after emergence. The levels of nitrogen were chosen with the middle level conforming to existing recommendations. All the potassium and sulphur treatments were applied before planting. As phosphorus was not under test, but was known to be essential for crop growth at the location, the whole trial received annual basal dressings of triple superphosphate to give 29.7 kg P per ha from 1966-67 to 1972-73 and, because of an error in the change to metric units, 32.5 kg P per ha from 1973- 74 to 1974-75. From 1975-76 the dummy treatment was used such that only half of the plots received the same level (32.5 kg P per ha), the remaining half being untreated in order to assess the residual effects.

Statistical analysis

The analysis was carried out at the Agricultural University of Norway for

all the years from 1966 to 1981 inclusive. A number of analyses with respect to yield, soil and leaf analysis, and other parameters were carried out at Rothamsted Experiment Station.

RESULTS

Yield of grain

Nitrogen

The analysis of variance for all 15 years shows a significant nitrogen x year interaction.

The linear effect (level 2 - level 0) on grain weight varied considerably from year to year, without any obvious trend. On average for all 15 years level 0 and level 2 gave about the same yield and there was no significant difference.

The quadratic effect, the difference between the mean level 0 and level 2, and level 1, is negative and about the same for all years. Over the 15 years mean level 1 has given significantly greater yields than the other two levels. From the 1976-77 season there is a tendency for level 2 to be lower than level 0. No significant interactions with other treatments were found.

Potassium

There was a tendency for the higher level of potassium to increase grain yields, on average by 117 kg per hectare, but this did not achieve significance in any year of the period, even when the rate was changed from 9.3 kg to 40 kg per hectare from the 1973-74 season onwards.

Sulphur

The yield response to sulphur for the first three years is shown in Table 3.

The effect of sulphur on grain yield was positive and significant over each of the first three years.

From the 1969-70 season the rate of sulphur was changed but the effect of 22.4 kg S over 11.2 kg S per hectare was

Table 2. The effect of nitrogen on the yield of grain. Kg ha^{-1} at 12.5% moisture

Year	N0	N1	N2	Linear effect	Quadratic effect	Mean	SE ±	Significance
1966-67	3839	3918	3936	97	-31	3898	103	<0.001
1967-68	5343	5284	5074	-269	-76	5234	110	<0.05
1968-69	5812	6412	6550	738	-231	6258	209	<0.01
1969-70	4041	4517	4584	543	-204	4381	113	<0.001
1970-71	4476	5283	5659	1183	-215	5139	178	<0.001
1971-72	4796	5846	5798	1001	-549	5480	116	<0.001
1972-73	6511	5964	5024	-1487	-197	5833	324	<0.001
1973-74	5589	6768	6830	1241	-599	6396	173	<0.001
1974-75	5843	7335	7100	1256	-864	6759	246	<0.001
1975-76	5816	6163	5286	-531	-612	5755	258	<0.001
1976-77	5579	5597	4789	-790	-413	5322	229	<0.01
1977-78	4106	4592	4117	10	-481	4272	222	<0.05
1978-79	3018	2824	2354	-664	-138	2732	221	<0.05
1979-80	3492	3442	2924	-568	-234	3286	242	<0.05
1980-81	4563	4861	3985	-578	-587	4470	217	<0.05
Mean	4855	5254	4934	79	-359	5014		
SE			±95					
Significance			<0.001					

Table 3. The effect of applied sulphur on the yield of grain. Kg ha^{-1} at 12.5% moisture

Year	S0	S1	S1-S0	Mean	SE ±	Significance
1966-67	3270	4526	1256	3898	119	<0.001
1967-68	5074	5393	320	5234	90	<0.01
1968-69	5659	6857	1198	6258	171	<0.001
Mean	4468	5090	622			
SE		±102				
Significance		<0.001				

not significant in any of the subsequent years.

Phosphorus

For the first nine years of the trial (to 1974-75) basal phosphorus at 29.6 to 32.5 kg per hectare was applied uniformly to all plots. From the 1975-76 season onwards the dummy treatment was used with only half the plots receiving further phosphorus fertilizer at 32.5 kg P per hectare. The effect of the residual versus the fresh phosphorus application during the period 1975-81 is shown in Table 4.

There was no significant difference between the treatments from 1975 to 1978. From the 1978-79 season onwards there was a positive and increasingly significant response to the freshly applied phosphorus on the yield of grain.

1000 grain weight

The 1000 grain weight was less affected by treatments than the yield was. The range of 1000 grain weight was from 334 g to 452 g, with an average of 398 g.

The linear effect of nitrogen was 9 g on average. The response varied with year and only in seven seasons out 14 was it significant. Sulphur had a significant positive effect in the second year when S1 was 15 g higher than S0. There was no further significant effect when the S0 level was changed to S2 in 1969-70.

Phosphorus was positively significant in the last two seasons with a 3 g and 20 g increasing in grain weigth respectively.

Table 4. The effect of phosphorus on the yield of grain. Kg ha^{-1} at 12.5% moisture

Year	P0	P1	P1-P0	Mean	SE ±	Significance
1975-76	5713	5797	85	5755	211	n.s.
1975-77	5204	5440	236	5322	187	n.s.
1977-78	4166	4377	210	4272	180	n.s.
1978-79	2465	2999	534	2732	180	<0.01
1979-80	2946	3627	681	3287	198	<0.01
1980-81	3560	5379	1819	4470	177	<0.001
Mean	4042	4636	594	4339		
SE		±146				
Significance		<0.001				

Table 5. The effect of nitrogen on 1000 grain weight, g.

Year	N0	N1	N2	Linear effect	Quadratic effect	Mean	SE	Significance
1966-67	435	435	431	3	-2	434	3.47	n.s.
1967-68	425	421	410	15	-4	419	6.46	n.s.
1968-69	435	443	452	17	1	443	5.03	<0.01
1969-70	369	365	375	6	7	370	7.52	<0.05
1970-71	368	391	405	37	-5	388	4.99	<0.001
1971-72	372	386	374	2	-13	377	4.15	<0.01
1972-73	333	333	336	3	2	334	8.60	n.s.
1973-74	432	460	464	32	-12	452	4.83	<0.001
1974-75	417	431	430	13	-8	426	8.03	n.s.
1975-76	391	389	379	12	-4	386	6.79	n.s.
1976-77	419	434	448	29	-1	434	8.47	<0.01
1977-78	358	356	356	1	1	357	3.14	n.s.
1978-79	369	369	370	1	1	369	1.69	n.s.
1979-80	366	369	380	15	5	372	5.32	<0.05
Mean	392	399	401	9	2	397		
SE			±1.5					
Significance			<0.001					

The effect of potassium was significant in the 1973-74 season only.

Other variables

Other variables recorded during the course of the trial included weight and number of cobs, number of plants at emergence, stand count at harvest, and shelling percentage.

No significant trend or consistent effect was found in any of these records. However, nitrogen affected the weight and number of cobs positively during the 1966-72 period, while in 1974-75 this effect was negative. The weight and number of diseased cobs were significantly reduced by nitrogen application in the 1973-75 seasons, on average from 13% (NO) to 6.4% (N2), but not in other seasons.

Sulphur application increased the weight and number of unshelled cobs significantly during the first three years (Table 6).

Potassium significantly increased the number of days to tasselling and silking, on average from 61 days to 67 days ($p<0.001$).

Leaf analysis

Analysis of variance of the nutrient content of leaves was carried out each year

Table 6. The influence of sulphur on the weight and number of unshelled cobs over the period 1966 to 1968

	S0	S1	Mean	SE ±	Significance
Kg per ha	5163	6174	5669	95	<0.001
Number per ha	41655	43945	42800	364	<0.001

from 1966-67 to 1972-73 for the elements N, P, K, Ca, Mg and S. Results from 1972-73 are not available.

Leaf nitrogen
Nitrogen fertilizer increased the percentage of leaf N significantly in all years except 1967-68. No interactions were consistent, or significant, except for the 1967-68 season when a negative N x K interaction was evident.

Leaf phosphorus
The phosphorus percentage in the leaf was positively and significantly affected by nitrogen application in the period 1966-67 to 1971-72. Only in 1967-68 was no significant response obtained.

Leaf potassium
Non consistent effect or significant interactions were found to the application of potassium fertilizer.

Leaf sulphur
During the first three years (1966-69) applied sulphur had a positive effect on the sulphur percentage of the leaves. However, in 1969-70 the response was negative and not significant in other years.

Leaf calcium and magnesium
No consistent effects were found in the leaf contents of calcium or magnesium to treatments.

Soil analysis
Soil chemical data for the period 1966-67 to 1971-72 are shown in Table 8.

DISCUSSION

At an early stage of fertilizer research in Zambia it was belived that only phosphorus was needed to improve yields on this soil type. Later evidence indicated that nitrogen was also necessary to maximize yields. Potassium was belived to be sufficient, but the need for sulphur was unclear. With this background the results obtained contribute to the understanding of the mechanism of soil fertility. Significant effects of nitrogen on yield of grain were obtained every year, although in both positive and negative directions. The linear effect of nitrogen interacted strongly with years, but varied without any clear trend. The quadratic effect, however, was consistently negative in all years, indicating that the highest level of nitrogen gave no additional yield response.

The yield of maize responded significantly to the application of sulphur at 11.2 kg per ha for the first three years. From the fourth season, it was decided to change the nil level to a S2 level of 22.4 kg per ha. However, no significant increase in yield between the two levels of sulphur was obtained. The results for the first three years clearly indicated that sulphur increases the yields of maize, and that the optimum application on this

Table 7. Mean percentage leaf nutrients

Nutrient	1966-72			1966-69				1969-72	
%	N0	N1	N2	K0	K1	S0	S1	S1	S2
N	2.35	2.55	2.70	2.53	2.50	2.39	2.51	2.57	2.58
P	0.25	0.27	0.27	0.26	0.26	0.25	0.24	0.27	0.28
K	2.43	2.47	2.48	2.45	2.47	2.55	2.60	2.37	2.38
S	0.16	0.17	0.18	0.17	0.17	0.14	0.16	0.19	0.18
Ca	0.25	0.26	0.26	0.25	0.25	0.23	0.23	0.27	0.27
Mg	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.18	0.18

Table 8. Mean soil data

		1966-67	1967-68	1968-69	1969-70	1970-71	1971-72	Mean
pH,	topsoil	5.5	5.4	5.4	4.8	4.9	4.8	5.1
	subsoil	4.9	-	-	-	4.8	-	4.9
Ex. Ca,	m.e./100 g,							
	topsoil	2.22	2.54	2.43	2.22	2.33	2.18	2.32
	subsoil	2.31	-	-	-	2.43	-	2.37
Ex. Mg.	m.e./100 g,							
	topsoil	1.43	1.84	1.77	1.66	1.63	1.43	1.63
	subsoil	1.68	-	-	-	2.07	-	1.88
Ex. K,	m.e./100 g,							
	topsoil	0.39	0.39	0.38	0.31	0.30	0.22	0.33
	subsoil	0.30	-	-	-	0.33	-	0.32
P, ppm,	topsoil	2.2	4.8	3.9	5.4	7.0	4.8	4.7
	subsoil	-	-	-	-	2.2	-	2.2
Org. C, %,								
	topsoil 0,94	-	-	-	0.71	-	0.83	
	subsoil 0.76	-	-	-	0.63	-	0.70	
Total N, %,								
	topsoil	0.063	-	-	-	0.059	-	0.061
	subsoil	0.008	-	-	-	0.056	-	0.057
CEC, m.e./100 g,								
	topsoil	5.96	-	-	-	7.07	5.93	6.32
	subsoil	8.33	-	-	-	8.38	-	8.36
BS, %,	topsoil	69	-	-	-	62	67	66
	subsoil	53	-	-	-	59	-	56

particular soil is less than 22 kg sulphur per hectare.

Potassium had a small but consistently positive effect in increasing yields, but this effect did not achieve significance in any of the 15 years.

Phosphorus was applied as a basal dressing equally on all plots until 1975-76, and on half the plots later on. The significant responses obtained clearly confirm earlier conclusisons that phosphorus fertilizer is necessary in each year for maximum response and that even after nine years the residual effect of 32.5 kg phosphorus per hectare annually is of short duration.

The stand count at harvest was significantly increased in 1969-72 by nitrogen and potassium but not during the first three years or in the 1973-74 season. Plant height seemed to be positively affected by nitrogen and phosphorus, while potassium slightly delayed maturity. The weight of diseased grains decreased significantly with nitrogen application. In some of the years the percentage of nitrogen and potassium in the leaves was significantly increased where N-, K- and S-fertilizers were applied. Weak and inconsistent N x K, N x S and K x S interactions were occasionally found.

The effects of fertilizer on the nutrient content of soils were not easily interpreted due to the high variability in the results. However, there seemed to be a

decrease in the content of nitrogen, phosphorus, potassium, magnesium and organic carbon with years. Both fertilizer and soil nutrients are absorbed by plant roots, and are lost by leaching and erosion. It is indicated that the amount of fertilizer applied is insufficient to maintain the nutrient level of the soil. The need for fertilizer in continuous cropping may increase as the soil becomes more and more depleted.

SUMMARY

A long-term fertilizer trial was started in the 1966-67 season at Magoye Regional Research Station in the Southern Province of Zambia. In the 1965-66 season the area had been opened up from virgin land and a uniformity trial conducted. The soil is a sandy clay loam, of medium acidity and cation exchange capacity, medium to rich in nutrients and with fairly high base saturation. The content of organic matter and total nitrogen was rather low.

The objective was to determine the extent to which nitrogen, potassium and sulphur, and eventually, phosphorus, would affect the yield of maize, and when possible responses would take place.

The linear effect of nitrogen was very variable and interacted strongly with year, while the quadratic effect was consistently negative. On average, the N1 level gave a significantly greater yield than the lower or higher levels.

The effect of potassium was positive, but not significant in any year. It was demonstrated that phosphorus fertilizer applied every year significantly increased the yield. During the first three years, 11.2 kg sulphur per hectare increased the yields significantly. No yield increase was found when the nil level was changed to 22.4 kg S per ha.

In some of the years, the percentage nitrogen and potassium was increased by N, K and S fertilizers, while in other years, no effect, or even negative effects were found. Soil analysis data were not clearly influenced by the fertilizer treatments. Most nutrients decrease with years, especially in the top soil as shown during the 1966-72 period.

ACKNOWLEGDMENTS

The authors thank the Norwegian Agency for International Development and the Agricultural University of Norway for financial and technical support, and all those officers and assistants who ensured the satisfactory conduct and continuity of the trial over so many years, particularly Mr. A.J. Prior, Principal Research Officer. Grateful acknowledgement is made to the Assistant Director of Research, Ministry of Agriculture and Water Development, Lusaka, for permission to publish this paper.

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MAIZE YIELD RESPONSE TO NITROGEN, PHOSPHORUS, POTASSIUM AND SULPHUR IN FERTILIZERS UNDER CONTINUOUS CULTIVATION IN THE CENTRAL PROVINCE OF ZAMBIA

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Tveitnes, S. & McPhillips, J. K. 1989. Maize yield response to nitrogen, phosphorus, potassium and sulphur in fertilizers under continuous cultivation in the Central province of Zambia. Norwegian Journal of Agricultural Sciences 3:191-199. ISSN 0801-5341.

In the first four years of the experimental period, it was found that the application of nitrogen increases the grain yield of maize while later on the effect is variable. The 1000 grain weight and number of cobs per hectare are affected by nitrogen fertilizer in a similar way to the grain yield.
There was a significant positive effect of phosphorus on grain yield, which was higher in the latter part of the research period. Sulphur has no effect on grain yield.
Nitrogen increases the leaf uptake of nitrogen, while both nitrogen and phosphorus increase the phosphorus content of the leaves. Phosphorus also increases the calcium content significantly. Neither potassium nor sulphur affect the concentration of leaf nutrient elements significantly.

Key words: Continuous cropping, maize, nitrogen, phosphorus, potassium, sulphur, Zambia.

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At the beginning of field experimental research in Zambia, it was believed that only phosphorus fertilizers were necessary in order to increase the yields of maize. Later it was demonstrated that nitrogen had a beneficial effect. Later still, several reports on the effect of sulphur demonstrated somewhat contradictory results as to the necessity of sulphur fertilizer application. There seemed to be little need of potassium fertilizer. However, many of the experiments conducted were short-term trials laid out on virgin land. In the years 1965-66 to 1967-68, three long-term fertilizer trials were set up at Misamfu, Magoye and Kabwe Regional Research Stations. The objective was to determine the long-term effects and validity of existing fertilizer recommendations. These sites cover the main agro-ecological zones from north to south in Zambia, and are sited on agriculturally important soil series. The Kabwe trial, which is reported here, was started in 1967-68.

METHODS

Site details

The Kabwe trial was carried out at the research station near Kabwe, at latitude 14°25'S and longitude 28°32'E. The mean annual rainfall during the period 1966-77 was recorded as 960 mm in 75 days, mainly from October to April, with a peak in December- January. The 1972-73 season had the lowest rainfall with 537 mm recorded in 55 days, while the highest rainfall occurred in the 1968-69 season with 1473 mm in 98 days.

Temperature data are not available prior to 1977, but the mean monthly temperatures during the 1977-80 period indicate that October is the warmest month with a mean of 24.5°C. During most of the season the temperatures are fairly stable, decreasing slightly from March onwards.

The soil at the experimental site belongs to the Milima Soil Series (Albic Ferric Luvisol in the FAO system).

The soil was analysed for pH and selected chemical properties, using the following analytical methods. pH was determined in 0.01 M $CaCl_2$, organic carbon by wet digestion (Walkley - Black method), exchangeable calcium and magnesium by atomic absorption, while potassium and sodium were measured on a flame photometer. Cation exchange capacity (CEC) was determined by leaching out any excess ammonium acetate with alcohol, and finally leaching with a sodium chloride solution to replace ammonia. In the subsequent distillation a boric acid indicator solution was used and titration was carried out with hydrochloric acid. Available phosphorus was determined by Bray's no. 1 method. Chemical soil data are presented in Table 9. The analyses were carried out at the soil laboratory of Mount Makulu Central Research Station, Chilanga.

Treatments

Nitrogen

N0	78 kg N per ha
N1	136 kg N per ha
N2	190 kg N per ha

Phosphorus

P0	Zero
P1	9.8 kg P per ha from 1967-68, and then 29.4 kg P per ha from 1973-74 to 1976-77.

Potassium

K0	Zero
K1	9.3 kg K per ha from 1967-68, and then 27.9 kg K per ha from 1973-74 to 1976-77.

Sulphur

S0	Zero
S1	9 kg S per ha.

The levels of nitrogen were chosen with the central point as the existing normal recommendations. This level had been found optimum in previous experiments in Zambia. The basal fertilizers were applied individually before planting in a band 10 cm to one side of the plant rows and about 10 cm deep. Sowing took place at the start of the rainy season, and varied from 15 December to 5 January depending on the season. The top dressing of nitrogen was banded on each side of the row.

Soil samples were taken from each plot before any fertilizer was applied and after ploughing and discing. Soil analysis data are also available from the years 1975-76 and 1976-77 from each plot at two depths, 0 - 15 cm and 25 - 45 cm.

The maize seed used was the hybrid SR 52 dressed with Agrosan. The crop was sown in 90 cm rows with 23 cm between plants in the row. Two seeds were sown 5 cm deep and then the soil and seed were dusted with 2.5% Aldrin before covering. Thinning to one plant per station was done when the plants were about 15 cm high. Weeds were controlled using the herbicide Atrazine at 2 kg per ha a few days after sowing and by

hand later on in the season. At about 4 weeks, or earlier if necessary all plants were treated with 5% granula DDT applied down the funnels to control stalkborer. The stover from the previous season was ploughed in at about 20-25 cm depths.

Statistics

A 3 x 2 factorial design of the four treatment factors was used. Treatment factor 1 has 3 levels, while treatment factors 2, 3 and 4 have 2 levels, including a nil level. Each of the four replications is divided into 4 blocks, with partial confounding of some of the 2 and 3 factor interactions (Cochran & Cox 1957). For a balanced plan, 3 replications are necessary but in fact 4 replications are used, with replication 1 repeated as replication 4. For this reason, a «correct» correction for block effects was not carried out. Instead, each figure was corrected with the difference between the average of the block in question and the replication. Since this correction may be too strong, a number of weaker corrections were tried, i.e. 1/3, 2/3, 8/10 and 9/10 of the above-mentioned correction, respectively.

As the average error within years (treatment x replication) for both experiments and both variables analysed was least by absolute (100%) correction, this correction has been used in the analysis of variance and calculations of means. In the analysis of variance the number of degrees of freedom for error was not reduced because of the block correction. This means that the calculated error could be 10 % too low.

RESULTS

Crop yields

Separate analyses of variance for the five-year period 1967-68 to 1971-72 and the five-year period 1972-73 to 1976-77 were carried out. The effect of the 4 factors and all interactions among these were tested against the interaction replication x treatment, and the interaction replication x treatment was tested against the interaction year x replication x treatment.

Table 1. The grain yield of maize and the response to nitrogen. Kg ha ^{1}

Year	Level of nitrogen N0	N1	N2	Linear effect	Quadratic effect	SE ±	Significance
1967-68	8220	8812	9210	990	-194	100	<0.001
1968-69	6682	6811	7192	510	252	136	<0.05
1969-70	5196	5569	6469	1273	527	136	<0.001
1970-71	6074	6234	6711	637	317	152	<0.05
1971-72	4761	4653	4667	-94	122	100	n.s.
1972-73	4911	4800	4813	-98	124	104	n.s.
1973-74	3537	3912	4553	1016	266	132	<0.001
1974-75	6671	6616	6662	-9	101	153	n.s.
1975-76	5766	5744	5650	-118	-70	192	n.s.
1976-77	4977	4959	5355	378	414	169	n.s.
Mean	5680	5811	6128	449	93		
SE		±81					
Significance		<0.001					

There was a tendency to decreasing yield levels throughout the research period, but with some variation from year to year.

Nitrogen

A significant effect of nitrogen fertilizer was demonstrated in the first four years, while in the fifth year, a negative but insignificant response was found. In the second five-year period, the effect of nitrogen application was very variable. There was a significant grain yield response in 1973-74 and in 1976-77, while in the other years of that period the effect of nitrogen on maize was insignificantly negative.

On average for the first five-year period the linear effect of nitrogen on grain yield was positive and significant ($p<0.001$), as was the interaction year x N. Over the second five-year period no such interaction was found. Only in 1973-74 was the nitrogen response considerable, especially for the first increment, otherwise the grain yield response to nitrogen was insignificant.

Phosphorus

The grain yield response to phosphorus was significant throughout the ten-year research period. The mean response to phosphorus was 434 kg grain per hectare over the first years, and 1295 kg per hectare over the second five-year period.

The interaction nitrogen x phosphorus was significant in both five-year periods, as shown in Table 3.

In the absence of phosphorus, the middle level of nitrogen had a negative effect on grain yield, while the higher N level had a significant positive effect. Where phosphorus was added the response to nitrogen was significantly greater, and then the middle level of nitrogen gave the best response.

Potassium

A significant effect of potassium was also shown. There was no significant interaction for year x potassium, and the response to potassium tended to vary from year to year, but without any clear trend. As a mean for the second five-year period, the grain yield response to potassium was somewhat higher than in the first five- year period.

Table 2. The grain yield of maize and the response to phosphorus fertilizers. Kg ha^{-1}

Year	Level of P				
	P0 ±	P1	P1-P0	SE	Significance
1967-68	8554	8940	386	82	<0.01
1968-69	6452	7338	886	111	<0.001
1969-70	5292	6197	905	111	<0.001
1970-71	5583	7097	1514	124	<0.001
1971-72	4354	5034	680	82	<0.01
1972-73	4492	5191	699	85	<0.01
1973-74	3531	4472	941	108	<0.001
1974-75	5971	7329	1358	125	<0.01
1975-76	4915	6727	1612	156	<0.001
1976-77	4191	6004	1813	138	<0.001
Mean	5334	6413	1079		
SE		±66			
Significance		<0.001			

Table 3. The effect of N x P interaction on grain yield of maize. Kg ha^{-1}

	1967-68 to 1971-72					1972-73 to 1976-77				
	P0	P1	Mean	SE ±	Signi-ficance	P0	P1	Mean	SE ±	Signi-ficance
N0	5891	6482	6187			4610	5737	5173		
N1	5577	7054	6316	81	<0.001	4363	6050	5206	91	<0.001
N2	6472	7227	6850			4887	5926	5407		
Mean	5980	6921	6451			4620	5904	5262		
SE	±66					±118				
Significance	<0.001					<0.01				

Table 4. The grain yield of maize and the response to potassium. Kg ha^{-1}

Mean	K0 ±	K1	K1-K0	SE	Significance
1967-68 to 1971-72	6350	6617	267	66	<0.05
1972-73 to 1976-77	5089	5436	347	53	<0.01

Sulphur

No main effects or any interactions with other nutrients were found.

1000 grain weight

The effect of nitrogen on the 1000 grain weight was similar to that on grain yield.

The difference between the zero and higher levels was significantly positive in eight of the ten years. The quadratic effect was negative in nine out of ten years, indicating that the highest N

Table 5. 1000 grain weight and the response to nitrogen. Kg ha^{-1}

Year	Level of nitrogen N0	N1	N2	Linear effect	Quadratic effect	SE ±	Signi-ficance
1967-68	513	530	530	17	-9	2.5	<0.001
1968-69	415	431	431	16	-8	4.6	<0.05
1969-70	440	452	463	23	-1	2.3	<0.001
1970-71	451	464	470	19	-7	2.1	<0.001
1971-72	361	367	359	-2	-7	2.6	n.s.
1972-73	361	367	360	-1	-7	3.0	n.s.
1973-74	392	408	429	37	5	3.5	0.001
1974-75	476	494	492	16	-10	4.6	<0.05
1975-76	472	498	504	32	-10	2.7	<0.001
1976-77	451	473	470	19	-25	5.9	<0.05
Mean	433	448	451	18	-6		
SE		±1.5					
Significance		<0.001					

Table 6. The effects of phosphorus, potassium and sulphur on the 1000 grain weight

	1967-68 to 1971-72	1972-73 to 1976-77
P0	462	438
P1-P0	2	10
SE±	1.36	1.23
Significance	n.s.	<0.001
K0	460	440
K1-K0	5	6
SE±	1.54	1.48
Significance	<0.01	<0.05

level resulted in a decrease in the 1000 grain weight. The effect of nitrogen varied significantly with years.

Phosphorus affected the 1000 grain weight during the 1972-73 to 1976-77 period only. Potassium increased the 1000 grain weight significantly in both five-year periods, as shown in Table 6, but the mean was less in the first than in the second five-year period.

Sulphur had no effect on the 1000 grain weight in the first five-year period. A significant effect was shown in the second five- year period (Table 6).

There was no significant nutrient x year interaction with regard to 1000 grain weight.

Other variables

The number of cobs per hectare was increased slightly owing to application of nitrogen in the first five-year period. However, during the period 1972-73 to 1976-77, there was a tendency towards a negative effect.

Phosphorus increased the number of cobs significantly in both periods. The effect varied from year to year, but was

Table 7. The effect of phosphorus on the number of cobs per 100 m^2

	Level of P				
	P0 ±	P1	P1-P0	SE	Significance
1967-68 to 1971-72	391	408	17	2.85	<0.001
1972-73 to 1976-77	363	408	45	4.56	<0.001

Table 8. Nutrient content of maize leaves. Per cent

	N0	N1	N2	P0	P1	K0	K1	S0	S1
N	2.38	2.58	2.69	2.54	2.57	2.54	2.56	2.54	2.57
P	0.26	0.27	0.28	0.25	0.28	0.27	0.27	0.27	0.27
K	2.08	2.09	2.06	2.08	2.07	2.07	2.08	2.06	2.09
S	0.17	0.18	0.17	0.17	0.18	0.17	0.18	0.17	0.18
Ca	0.47	0.47	0.48	0.46	0.49	0.47	0.47	0.47	0.48
Mg	0.16	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.17

greater in the second than in the first five-year period.

Other variables recorded during the course of the trial included number of plants at emergence, stand count at harvest and shelling percentage. No significant trend was found in any of these records.

Leaf analysis

Leaf analysis results are available from the 1967-68 to 1969-70 seasons and from the 1972-73 to 1976-77 seasons. Analyses of variance were carried out for the various elements.

Leaf nitrogen

Nitrogen significantly increased the content of nitrogen in the leaves of maize significantly ($p<0.001$) over all eight years. Although the level varied from year to year, the nitrogen response was consistent during the whole period. None of the other nutrients applied affected the leaf nitrogen percentage significantly.

Leaf phosphorus

With the exceptions of the 1967-68 and 1973-74 seasons, nitrogen fertilizers increased the leaf phosphorus percentage, and the effect was significant ($p< 0.001$) on average for all eight years.

While phosphorus fertilizer increased the phosphorus content of the leaves ($p<0.01$), neither potassium nor sulphur fertilizers had any effect.

Leaf potassium
None of the nutrients applied had any significant effect on the potassium content of maize leaves.

Leaf sulphur
Sulphur fertilizer had a positive but insignificant effect on the content of leaf nutrients throughout the experimental period.

Leaf calcium
Of three nutrients applied as fertilizer only phosphorus increased the content of calcium in the leaves significantly.

Leaf magnesium
Applied phosphorus and sulphur tended to increase the magnesium percentage, whilst the potassium had little effect.

Soil analysis results
Soil acidity
During the experimental period, the pH dropped considerably from 6.0 in the top 20 cm layer at the start of the experiment in 1967-68, to 4.6 in 1976-77. The change in pH in the 20-40 cm soil layer was from 5.8 to 5.5 only. The use of fertilizers affected the soil pH only insignificantly in the course of this experi-

Table 9. Chemical soil variables

	1966-67		1975-76		1976-77	
	0-15 cm	25-45 cm	0-15 cm	25-45 cm	0-15 cm	25-45 cm
pH ($CaCl_2$)	6.0	5.8	4.7	5.5	4.6	5.5
Organic C, %	-	-	0.36	0.28	0.44	0.37
Exch. Ca, m.e./100 g	1.68	1.17	1.26	1.76	1.03	1.94
Exch. Mg, m.e./100 g	0.46	0.42	0.21	0.35	0.24	0.73
Exch. K, m.e./100 g	0.10	0.09	0.05	0.07	0.13	0.13
CEC, m.e./100 g	2.76	2.30	2.09	2.25	1.92	2.83
BS, %	78	73	74	90	72	96
P, ppm	-	-	7.3	5.1	7.3	5.1

ment. Nitrogen fertilizer reduced pH by 0.1 - 0.3 pH-units, an effect far less than the overall effect of cultivation.

Organic carbon
There was no indication that the use of fertilizers affected the organic carbon content significantly. However, there was a tendency toward an increase in the carbon percentage from 1974-75 to 1976-77, probably because of the incorporation of crop stalks and root residues in the soil.

Total nitrogen
There was a negligible change in the nitrogen content of the soil after application of fertilizers. The level of total nitrogen was very low, only 0.03 - 0.05%.

Exchangeable cations
The content of calcium and magnesium tended to decrease throughout the experimental period, but increased in the subsoil. Changes caused by the use of fertilizers were inconsistent. The content of exchangeable potassium was very low, and was not affected by fertilizer application.

Cation exchange capacity (CEC)

The cation exchange capacity of the topsoil decreased during the experimental period, while there was a tendency toward an increase in the subsoil layer. The changes in the CEC caused by the use of fertilizers were small and inconsistent.

Base saturation percentage
There was a tendency toward a decrease in the base saturation percentage in the topsoil layer, and an increase in the subsoil during the experimental period. No systematic changes attributable to application of fertilizers were found.

Phosphorus
No consistent changes in the content of phosphorus took place as a result of fertilizer application throughout the experimental period.

DISCUSSION

The objective of the trial was to determine to what extent nitrogen, phosphorus, potassium and sulphur would affect the grain yield of maize, and to find out whether it would be possible to maintain the yield level under continuous maize cropping.

The nitrogen effect on grain yield was clear and significant during the first four years of the experimental period, while later on the grain yield response was variable. This may have been due to a general deterioration of the growth conditions, probably caused by several factors, e.g. lack of particular micronutrients, deterioration of soil physical conditions and also variation in climatic conditions. Also, in previous experiments in the region, nitrogen has had variable effects on crop yield. Thus, Rattray (1949) demonstrated a response to nitrogen fertilizer, while experiments with urea (McPhillips 1983) showed no beneficial effect on crop yield in the absence of sulphur.

Pawson (1952) indicated that phosphorus responses were mainly limited to areas in the Southern Province. However, the grain yield response to phosphorus was significant during the whole ten-year period, being greater in the second five-year period following an increase in the level applied, indicating that phosphorus is highly important on such sandy soils.

There was also an indication that the effect of potassium was better in the latter period, although the trend was not very clear.

Sulphur did not affect grain yield, but as a mean of the second five-year period the 1000 grain weight was significantly increased. Previous research on sulphur in Zambia indicated that this element is not deficient in the fertile

soils of Central, Eastern and Southern Provinces (Annual Report of the Research Branch 1954). Sulphur deficiency has been revealed on less fertile soils (McPhillips 1983).

Nitrogen increased the nitrogen concentration in maize leaves, while phosphorus clearly increased the content of both nitrogen and phosphorus in the leaves.

As in the Magoye experiment (Tveitnes & McPhillips 1989), the effect of fertilizers on the nutrient content of soils is difficult to interpret, owing to high variability in the results. In the topsoil there was a tendency for calcium, magnesium, cation exchange capacity, and the base saturation percentage to decrease slightly throughout the experimental period, presumably the result of the removal of nutrients by the crop, since the major part of the plant roots is found in the top layer. A certain leaching down of nutrients may also have taken place, since there is a tendency toward an increase in some of the soil variables in the subsoil.

SUMMARY

The effects of nitrogen, phosphorus, potassium and sulphur fertilizers are being studied in a long-term trial initiated in 1967-68 at Kabwe Regional Research Station in the Central Province of Zambia.

The application of nitrogen increased the grain yield of maize during the first four years of the experimental period, while later on the effect was variable. The 1000 grain weight and number of cobs per hectare were affected by nitrogen fertilizer in a similar way to the grain yield.

The positive effect of phosphorus on grain yield was significant and was higher in the latter part of the research period. Sulphur had no effect on grain yield.

Nitrogen increased the leaf uptake of nitrogen, while both nitrogen and phosphorus increased the phosphorus content of the leaves. Phosphorus also increased the calcium content significantly. Neither potassium nor sulphur affected the concentration of leaf nutrient elements significantly.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the efforts made by all research officers and assistants who ensured the continuity of the trial. The authors also thank the Norwegian Agency for International Development and the Agricultural University of Norway for financial and technical support towards the publication of this paper. Grateful acknowledgement is made to the Assistant Director of Research, Ministry of Agriculture and Water Development, Lusaka, for permission to publish this paper.

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EFFECTS OF PHOTOPERIOD, TEMPERATURE AND VERNALIZATION ON FLOWERING AND GROWTH IN HIGH-LATITUDE POPULATIONS OF RED CLOVER

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Lunnan, T. 1989. Effects of photoperiod, temperature and vernalization on flowering and growth in high-latitude populations of red clover. Norwegian Journal of Agricultural Sciences 3:201-210. ISSN 0801-5341.

Seedlings were grown for two to five months at 8-h and 24-h photoperiods and at three or five temperature levels of between 9°C and 24°C. Both shoot and root growth are on average from two to four times greater at 24-h, and the effect is most pronounced at low temperatures. Ecotypes from 71 and 65°N are more stimulated by the longer photoperiod than the ecotype from 61°N. Low temperature is favourable for dry matter production because of the low rate of phenological development and thereby increases stem and leaf formation. Only plants grown at long-day periods flower. The highest proportion of flowering plants is found at low temperatures, and in the southernmost ecotype. An increase in temperature from 9°C to 24°C accelerates flowering considerably. Vernalization enhancement of plant growth and development in one of the two experiments, has ultimately been considered as spurious.

Key words: *Trifolium pratense*, ecotypes, flowering, growth, photoperiod, temperature, vernalization.

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Red clover (*Trifolium pratense* L.) is the most common forage legume in Norway. It requires long-day conditions to flower, and northern ecotypes require longer photoperiods than southern ones (Julén 1977). Red clover has normally no vernalization requirement for flowering (Aitken 1964), although stimulating effects of a low temperature treatment are reported for some ecotypes (van Dobben 1964, Kaneko et al. 1968, Bula 1969). The object of the present experiments was to study the effects of photoperiod, temperature and vernalization on flowering and growth of Scandinavian red clover populations.

MATERIAL AND METHODS

Two experiments were carried out in the phytotron of the Agricultural University of Norway with seedlings of diploid red clover originating from different latitudes:

Plant material	Latitude °N
'Holt'	71
'Bjursele'	65
'Molstad'	61

'Holt' is a population collected in the neighbourhood of Tromsø, 'Bjursele' is a local Swedish cultivar from Västerbotten and 'Molstad' is a local cultivar from southeastern Norway. In these experiments they will be called ecotypes.

In Exp. 1 the seedlings were raised in a greenhouse under warm, long-day summer conditions. At the start of the experiment (August 5th 1986) sowing dates and number of visible leaves, unifoliate included, on the main axis were:

Ecotype	Vernalized	Non-vernalized	No. of leaves
'Holt'	25 June	13 July	2.1
'Bjursele'	25 June	13 July	2.7
'Molstad'	25 June	13 July	2.8
No. of leaves	3.1	2.0	

Vernalized plants were larger than the non-vernalized ones. 'Holt' germinated late and had the lowest number of leaves at the start of the experiment.

In Exp. 2 the seedlings were raised in a greenhouse during the autumn 1987 at a 12-h photoperiod and at 15-21°C. 'Holt' was sown first in order to have the plants at the same size at the start of the experiment. Sowing dates and leaf number on the main axis at the start of Exp. 2 (December 22nd 1987) were:

Ecotype	Vernalized	Non-vernalized	No. of leaves
'Holt'	2 Oct	15 Oct	9.5
'Bjursele'	9 Oct	4 Nov	8.8
'Molstad'	12 Oct	6 Nov	8.5
No. of leaves	8.5	9.5	

The plants of Exp. 2 were much larger and had developed more leaves than those of Exp. 1. A few 'Molstad' seedlings had started stem elongation before being inset in the phytotron in Exp. 2. This was not expected at a 12-h photoperiod, but an accidental prolongation of the photoperiod might have been due to very weak light from a neighbouring greenhouse.

A factorial split-split-plot design was used. In each temperature cabinet plants of the three ecotypes, vernalized or non-vernalized, were randomized within the photoperiod treatments. Each ecotype was represented by eight single plants in Exp. 1, and five in Exp. 2, per combination of vernalization treatments, temperature and photoperiod (as shown in Table 1). The plants were kept in daylight phytotron compartments for 8 h and at the same temperatures in adjacent rooms with darkness or with weak light from incandescent lamps (photosynthetic photon flux density, PPFD, of about 2 µmol m^{-2} s^{-1}, 3.6 Wm^{-2}) for the 24-h photoperiod treatment. Thus, the daily amount of radiant energy was nearly identical for the two photoperiod treatments. Daylight was supplemented by HPI/T-lamps (PPFD of about 50 µmol m^{-2} s^{-1}, 18 Wm^{-2}, at plant level) during the winter. In Exp. 1 the plants were vernalized at 6°C for 21 days, in Exp. 2 at 5°C for 14 days, at an 11-h photoperiod before being inset in the phytotron.

The plants were grown singly in plastic pots of 0.5 l capacity with a peat-soil mixture. They were not inoculated with *Rhizobium* bacteria, but fed nitrogen by a 13-6-16 NPK fertilizer. Mites (*Tetranychus* spp.) were controlled using insecticides, but might have caused some harm at the highest temperatures in both experiments.

Long-day plants were harvested singly as they started to bloom. Plants that failed to bloom were harvested at the end of each experiment, first at the highest temperatures. Plants with stems were then controlled for flowering, and

most of them were in a vegetative stage of growth. Short-day plants were harvested at about the median harvest time of long-day plants at the same temperature (Table 1).

Table 1. Growing time in the phytotron. Averages of ecotypes, vernalization treatments and parallels

Temperature, °C:	9	12	15	18	21	24
Exp. 1						
8-h photoperiod		- 116		- 111		- 100
24-h "		- 142		- 124		- 91
Exp. 2						
8-h photoperiod	139	108	92	66	48	-
24-h "	146	110	94	78	60	-

The number of leaves, fresh or wilted, was counted, and the green leaflet area was measured on a LI-COR LI-3000 leaf area meter. The dry matter of leaflets, petioles and stems with flower heads was recorded separately after drying at 80°C for 48 h. In Exp. 1 etiolated regrowth after 28 days at 18°C in darkness was measured on 50% of the plants. On the remaining plants the roots were washed and dried, as was done with all plants in Exp. 2. On flowering plants the number of stems, stem branches, internodes >0.5 cm and stem length were recorded in both experiments.

The mean values over parallels were subjected to a factorial analysis of variance. The effects of temperature, photoperiod, vernalization, ecotype, two-factor interactions and temperature x photoperiod x ecotype were tested against a pooled error consisting of the remaining three- and four-factor interactions.

RESULTS

A. Flowering

Only plants grown under continuous light flowered. In Exp. 1 'Molstad' had the highest proportion of flowering plants at all temperatures, and 'Holt' the lowest proportion (Fig. 1). In Exp. 2 all 'Molstad' plants flowered through the whole temperature range. A high proportion of 'Holt' and 'Bjursele' flowered at 9°C, with a high proportion of 'Bjursele' also flowering at 12 and 15°C, whe-

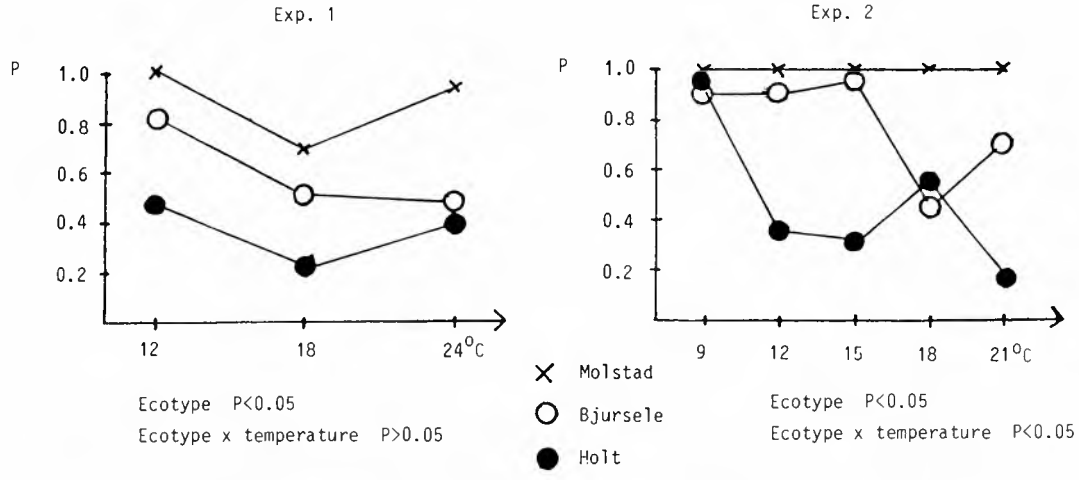

Figure 1. Proportion (P) of flowering plants grown at the 24-h photoperiod and at different temperatures. Exp. 1 (left), and Exp. 2 (right)

reas more 'Holt' plants remained vegetative above 9°C.

The vernalization treatment enhanced the proportion of flowering plants in Exp. 1, but not in Exp. 2:

	Exp. 1	Exp. 2
Non-vernalized plants	0.49	0.77
Vernalized plants	0.72	0.72
P value	<0.05	>0.05

The rate of development to first flowering increased with temperature according to the heat sum concept; thus giving a close and linear relationship between the rate of development and temperature:

Exp. 1: $1/D = 0.0017 + 0.00050\ T$, $R^2 = 0.998$ ($P<0.01$)
Exp. 2: $1/D = -0.0037 + 0.00121\ T$, $R^2 = 0.994$ ($P<0.01$)

where D is days from inset into the phytotron to first flowering and T is temperature in °C.

'Molstad' flowered first, and 'Holt' last in both experiments (Table 2). There was no significant temperature x ecotype interaction. The inter-plant variation in flowering time was large.

Table 2. Time in days from inset in the phytotron to flowering for the ecotypes at the24-h photoperiod. Averages of temperature and vernalization treatments

Ecotype	Exp. 1	Exp. 2	Range
'Holt'	111	108	36-231
'Bjursele'	103	90	22-223
'Molstad'	89	63	15-202
P value	<0.05	<0.01	

In Exp. 1, vernalized plants on average flowered after 90 days as compared with 111 for non-vernalized plants ($P<0.01$). In Exp. 2 the opposite relationship was observed with mean values of 92 and 82 days, respectively ($P>0.05$).

B. Growth of flowering plants

There was no significant effect of temperature or vernalization on the length of the flowering stem at flowering. 'Holt' had the shortest stems, and 'Molstad' the longest ones in both experiments, but the inter-plant variation was large (Table 3).

Table 3. Length of the first flowering stem (cm) in three ecotypes grown under continuous light. Averages of temperature and vernalization treatments

Ecotype	Exp. 1	Exp. 2	Range
'Holt'	69	60	42-106
'Bjursele'	88	81	36-134
'Molstad'	109	103	53-168
P value	<0.05	<0.01	

The number of elongated internodes on the flowering stem was not significantly affected by either temperature or vernalization. 'Holt' had most internodes in Exp. 2 (Table 4).

Table 4. Number of internodes on the flowering stem of three ecotypes grown at the 24-h photoperiod. Averages of temperature and vernalization treatments

Ecotype	Exp. 1	Exp. 2
'Holt'	11.2	15.5
'Bjursele'	10.5	13.7
'Molstad'	11.0	11.3
P value	>0.05	<0.05

The total number of stems on the flowering plants was highest at the lowest temperature as shown below:

Temperature °C:	9	12	15	18	21	24
Exp. 1	-	4.4	-	2.1	-	1.9
Exp. 2	7.1	3.9	4.5	3.1	3.1	-

Also, the number of stem branches per plant was highest at the lowest temperature. In Exp. 2 the average number at 9°C was 17.2 branches, with a decline to 2.1 at 21°C. In Exp. 1 there was a decline from 3.6 branches at 12°C to 2.5 at 24°C. There was no consistent difference between ecotypes in numbers of stems and branches. Vernalization had a positive effect on these characters in Exp. 1, but not in Exp. 2.

The proportion of stems in shoot DM was highest at low temperatures (Fig. 2). The stem content in shoot DM decreased by 1.1%- unit per degree centigrade increase in temperature.

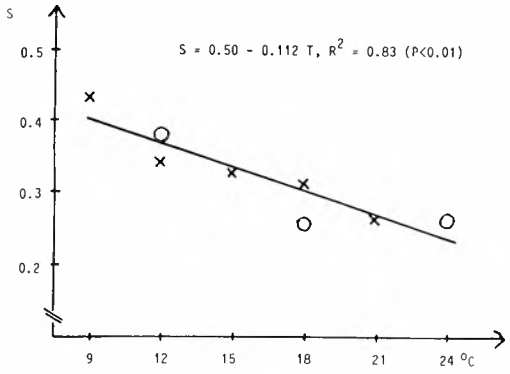

Figure 2. Proportion of stem matter in shoot DM (S) of flowering plants grown at the 24-h photoperiod and different temperatures (T °C). Average of ecotypes and vernalization treatments. o Exp. 1, x Exp. 2

Among the flowering plants 'Molstad' had the highest and 'Holt' the lowest stem matter content in shoot DM (Table 5).

Table 5. Proportion of stem matter in shoot DM of three ecotypes grown under continuous light. Averages of temperature and vernalization treatments

Ecotype	Exp. 1	Exp. 2
'Holt'	0.23	0.25
'Bjursele'	0.26	0.33
'Molstad'	0.40	0.43
P value	<0.05	<0.01

C. Overall growth

In table 6 it can be seen that the rate of leaf appearance was highest at the 8-h photoperiod ($P<0.01$). At the 24-h photoperiod the ecotypes had a similar rate, while 'Molstad' had the highest at the 8-h photoperiods in both experiments ($P<0.01$).

Table 6. Mean rate of leaf appearance of three ecotypes grown at 8- and 24-h photoperiods. Averages of temperature and vernalization treatments, and 2 experiments, and for the period inset to harvesting

Ecotype	Number plant^{-1} day^{-1} 8 h	24 h
'Holt'	1.29	1.17
'Bjursele'	1.51	1.27
'Molstad'	1.97	1.00
Mean	1.59	1.14

Leaf appearance was most rapid at 18°C in Exp. 1, and at 15°C in Exp. 2. The values shown below are the averages of photoperiods, ecotypes and vernalization treatments.

	Leaf appearance, number plant^{-1} day^{-1}					
Temperature, °C	9	12	15	18	21	24
Exp. 1	-	0.86	-	1.23	-	1.17
Exp. 2	1.62	1.79	1.97	1.68	1.16	-

In Exp. 1 vernalized plants developed 1.26 leaves day^{-1}, as compared with 0.91 for non-vernalized plants. In Exp. 2 the relationship changed to 1.49 and 1.80 leaves day^{-1}, respectively.

The rate of leaf area expansion from inset into the phytotron to harvest was higher at the 24-h than at the 8-h photoperiod (Fig. 3). In both experiments the difference in leaf area expansion attributable to photoperiod was most pronounced at low temperatures ($P<0.01$).

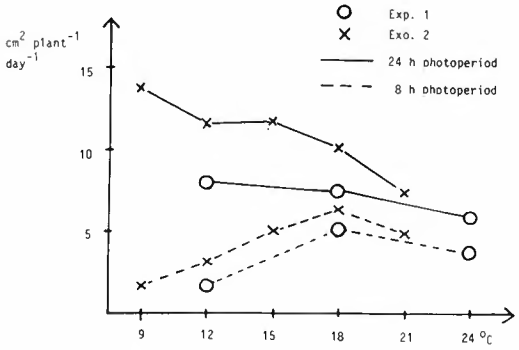

Figure 3. Rate of leaf area expansion at the 8- and 24-h photoperiods in two experiments. Averages of ecotypes and vernalization treatments

There were significant differences between the ecotypes in the leaf area expansion in both experiments, particularly in Exp. 1. As can been seen from Table 7 'Holt' showed the lowest score.

Vernalized plants developed a larger leaf area in Exp. 1 with a mean rate of 6.1 cm^2 plant^{-1} day^{-1} as compared with 4.4 for non-vernalized plants ($P<0.01$). In Exp. 2 the values were 7.9 and 7.1 cm^2, respectively ($P>0.05$).

The shoot growth rate was higher at the 24-h than at the 8-h photoperiod ($P<0.01$), and a temperature x photoperiod interaction was evident in both experiments ($P<0.01$). The difference in shoot growth rate between the 8- and 24-h photoperiods was great at low temperatures, and decreased with increasing temperatures. The shoot growth rate at low temperatures was higher in Exp. 2 than in Exp. 1 (Fig. 4).

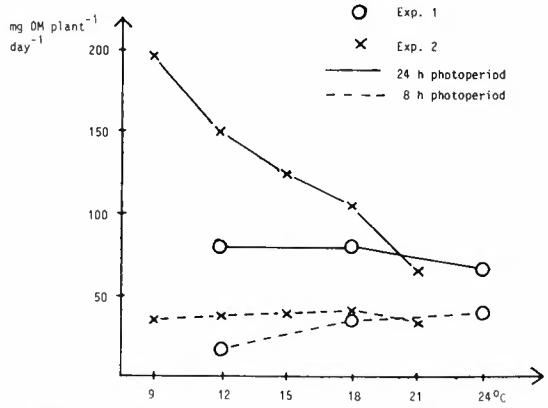

Figure 4. Shoot growth rate at the 8- and 24-h photoperiods in two experiments. Averages of vernalization treatments and three ecotypes

Table 7. Rate of leaf area expansion in cm^2 plant^{-1} day^{-1} of three ecotypes. Averages of temperature and vernalization treatments

Ecotype	Exp. 1	Exp. 2
'Holt'	2.5	6.0
'Bjursele'	5.3	8.4
'Molstad'	8.0	8.2
P value	<0.01	<0.01

At low temperatures the root growth rate was higher in Exp. 2 than in Exp. 1, and in particular at the 24-h photoperiod (Fig. 5). The positive effect on root growth of a prolonged photoperiod was reduced to near zero at high temperatures in both experiments ($P<0.05$).

The ecotypes responded differently to duration of photoperiod ($P<0.05$ for shoots and $P<0.01$ for roots). Thus, 'Molstad' grew best at an 8-h photoperiod, while 'Bjursele' and 'Holt' were relatively much more stimulated by continuous light (Table 8).

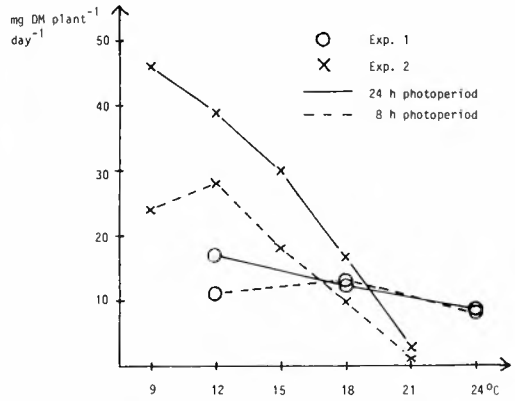

Figure 5. Effect of temperature on root growth rate at the 8- and 24-h photoperiods in two experiments. Averages of ecotypes and vernalization treatments

Table 8. Shoot and root growth rate (mg plant^{-1} day^{-1}) at the 8-h photoperiod and relative rate at 24 h (24 h/8 h) for three ecotypes of red clover. Averages of temperature and vernalization treatments, and two experiments

Ecotype	Shoot		Root	
	8 h	24 h/8 h	8 h	24 h/8 h
'Holt'	21	3.8	8	2.9
'Bjursele'	30	3.8	12	2.0
'Molstad'	47	2.3	19	0.6

In Exp. 1 the shoot growth rate increased from 43 mg for non-vernalized to 61 mg plant^{-1} day^{-1} for vernalized plants ($P<0.01$). In Exp. 2 no vernalization effect was found with values of 83 and 81 mg, respectively. Root growth rate increased by vernalization in Exp. 1, whereas the opposite occurred in Exp. 2:

	mg DM plant^{-1} day^{-1}	
	Exp. 1	Exp. 2
Non-vernalized plants	9	24
Vernalized plants	14	18
P value	<0.01	<0.01

In Exp. 1 etiolated regrowth was well correlated with root growth rate. Thus, the largest average value was 113 mg plant^{-1} for plants grown at 12°C, and the smallest 57 mg at 24°C. Small 'Holt' plants from short days had poor etiolated regrowth in spite of a low shoot/root ratio.

The average shoot/root ratio at harvest was highest at high temperatures in both experiments ($P<0.01$):

Temperature °C:	9	12	15	18	21	24
Exp. 1	-	3.6	-	5.0	-	6.2
Exp. 2	3.2	3.1	3.8	4.8	6.1	-

At an 8-h photoperiod the ecotypes had similar shoot/root ratios of 3.1-3.3. However, as can be seen in Table 8, the ecotypes responded differently ($P<0.01$) to an increase in photoperiod to 24 h. The mean values at the 24-h photoperiod were 4.7, 5.6 and 7.6 for 'Holt', 'Bjursele' and 'Molstad', respectively.

DISCUSSION

High-latitude populations of red clover require photoperiods of 16-18 h in order to flower (Schulze 1957, van Dobben 1964), as compared with 12-14 h for early-flowering, low-latitude types (Ludwig et al. 1953, Bowley et al. 1987). Van Dobben (1964) found a vernalization requirement in a plant of Norwegian wild red clover taken from Bøvertun (latitude 61°N, altitude 680 m). Clones of this plant remained vegetative when grown at a constant temperature of 25°C under continuous light. In the present experiments a flowering and growth promoting effect of vernalization was found in Exp. 1, but not in Exp. 2. This difference might have been due to strong interplant competition for light. Vernalized plants were larger at the start of Exp. 1, and the weaker growth and flowering of the non-vernalized plants in Exp. 1

might have been due to shading. Low light levels are known to reduce flowering and to increase the time needed to reach that stage (Bula 1960, Frey & Nösberger 1980). The results from Exp. 2 indicate that vernalization has a very small effect on growth and flowering in these ecotypes. This concurs with field observations of some few flowering plants in the seeding year even of very late flowering ecotypes after warm summers (Wexelsen 1952).

The highest proportion of flowering plants occurred at low temperatures (Fig. 1). At constant high temperatures some plants probably became too weak to flower because of high respiration and shading from neighbouring plants. The photoperiod requirement and the reduced flowering of high-latitude red clover populations at high temperatures appear to be quite similar to those of timothy (*Phleum pratense* L.), as shown by Heide (1982).

The time to flowering decreased considerably with increasing temperature. The later flowering in Exp. 1 than in Exp. 2 was due to the introduction of smaller plants at the beginning of the experiment. The number of elongated internodes on the flowering stem was positively correlated with the time to flowering in both experiments. The earliest plants of all ecotypes flowered at 6-7 internodes at all temperatures, while the latest ones had about 20. The average increase in internode number per day of delayed flowering was 0.070 in Exp. 1 and 0.107 in Exp. 2. This concurs with the results of Hawkins (1953). Early flowering plants or cultivars have less elongated internodes than late flowering ones.

Fagerberg (1988) studied the phenological development in stands of red clover and found that flowering took place after 900 degree days (base temperature 0°C). A decrease in daily mean temperature from 16 to 15°C would then cause a delay in flowering of 3.8 days. In the present experiments a decrease from 16 to 15°C would cause a delay of 5.6 and 5.3 days in Exps. 1 and 2. The higher number of days found in the present experiments may be due to a slower development of seedlings compared with overwintered plants in the field, and to light levels.

Some plants developed very long stems. 'Molstad' had plants longer than 150 cm, and the average stem height at flowering of this ecotype was more than 1 m in both experiments (Table 3). This shows that red clover is well adapted to mixtures with tall grasses.

The average growing time and the total amount of photosynthetic active radiation were almost the same for the 8- and 24-h photoperiod treatments. A higher number of leaves per plant was found under short days (Table 6). This is possibly due to promotion of leaf formation under short days, while long days stimulate leaf expansion and the formation of stems and flowers. The size of individual leaves, and the total leaf area as well as the total shoot growth rate, were larger under long days, and this effect was most pronounced at low temperatures (Figs. 3 and 4). Because of stem elongation the leaves are distributed in a higher canopy giving a better light penetration and interception in the stand. Consequently, red clover has been found to accumulate dry matter fastest during stem elongation (Frey & Nösberger 1980, Bowley et al. 1988). Similar effects of long days on growth have been found in high-latitude *Poa pratensis* L. by Heide et al. (1985). They also applied GA_3 which compensated for long days, and they suggested that the longday effects on growth were mediated by changes in endogenous gibberellins.

At 8-h photoperiods the northernmost ecotype 'Holt' had very weak growth (Table 8). Also at this very short day length ecotype differences in photoperiodic requirement for growth cessation were reflected. 'Molstad' had the most vigorous growth, 'Bjursele' was intermediate, and all the ecotypes had al-

most the same shoot/root ratios of about 3.2 at short days. The decline in the root growth rate of 'Molstad' with increased photoperiod (Table 8) might be caused by the strong stem growth and flowering in this ecotype. In red clover there is a negative relationship between flowering in the seeding year and winter survival (Smith 1963).

The temperature effect on growth was made up by its influence on the growing time, which was shortened by rising temperatures (Table 1) and by specific effects on morphological development. Plants at long days and high temperatures flowered early, and had developed a small leaf area at flowering. The mean growth rate was higher at low temperatures (Fig. 4). This might partly be due to the fact that plants at low temperatures had a larger leaf area during most of their long growing time. Furthermore, the higher stem content of shoot DM at low temperatures (Fig. 2) may have contributed to the higher shoot growth rate at low temperatures. In Exp. 2 plants with a long growing time also had better light conditions, as the natural irradiance increased rapidly during springtime. Probably the optimum temperature for growth would have been higher if all plants had been harvested at the same time in a short-term experiment.

The mean rate of leaf appearance was highest at 18°C in Exp. 1, and at 15°C in Exp. 2. The mean rate of shoot formation decreases with rising temperature, while the rate of leaf formation per stem increases. Accordingly, the expression number of leaves per plant per day, which is the product of these two terms, may get a maximum value at intermediate temperatures. This will also be in agreement with the small effects on leaf area and on shoot DM production in red clover by day/night temperatures from 14/9°C to 26/21°C, as found by Jelmini & Nösberger (1978).

The present experiments support the view that red clover is a simple long day plant. No vernalization or short-day treatment is needed for it to flower even in these high-latitude ecotypes. Constant high temperatures reduced the proportion of flowering plants in the two northernmost ecotypes; this might be a constraint for seed production in southern areas. However, the same reduction in flowering was found in timothy cultivars from northern Norway (Heide 1982), and these are good seed producers in southern Norway (Skaare & Hillestad 1973). The demand for a long photoperiod is probably more important. If high-latitude populations are grown for seed at places with photoperiods shorter than 17-18 h, only parts of the plants will flower. This will make an unfavourable selection towards early flowering plants with reduced winter hardiness.

ACKNOWLEDGEMENTS

The author thanks Prof. Dr. Birger Opsahl for his help during the experimental period and with the manuscript. Thanks are also extended to Prof. Dr. Arne O. Skjelvåg for positive criticism of the manuscript.

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